JOURNAL OF DAIRY SCIENCE

MAY, 1957

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ANNUAL MEETING
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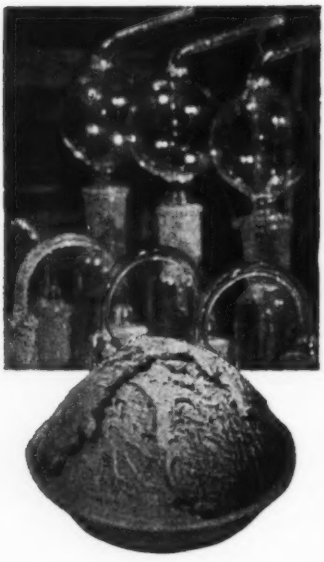

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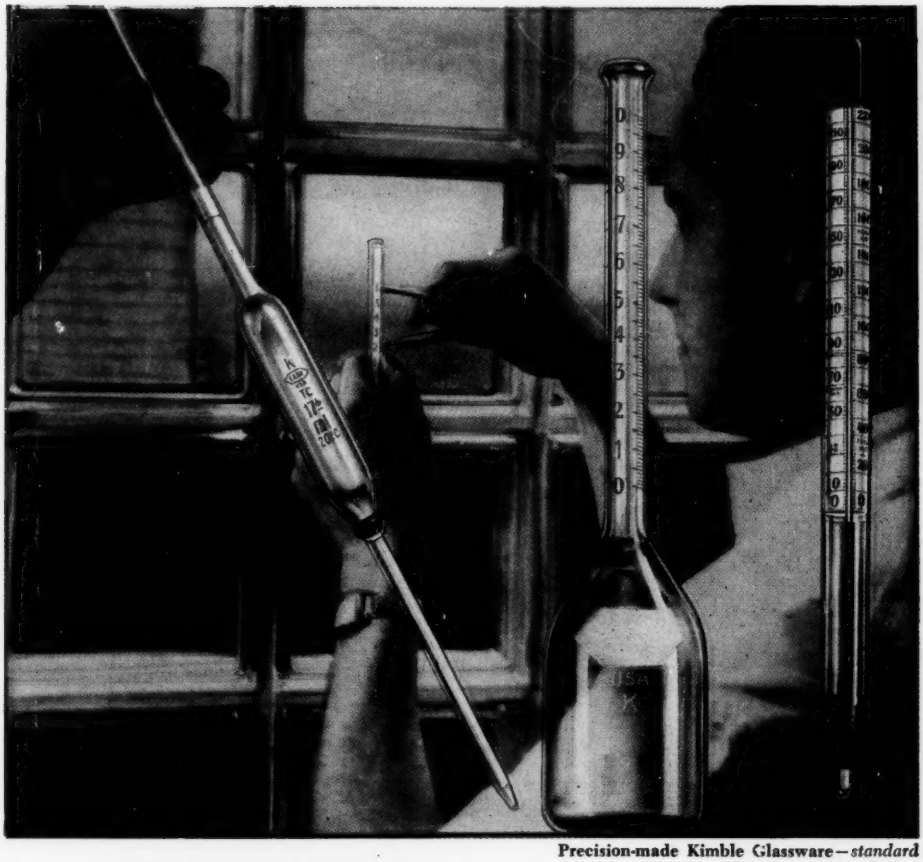

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PEOPLE and EVENTS
in the Dairy Science World

Beatrice Promotions

BROWN W. CANNON, Denver, Colorado, has been promoted to the newly created position of Western Regional Vice-President of Beatrice Foods Co. He will head up five new districts in the Rocky Mountain and Pacific Coast areas.

Advancement of five division managers to posts as district managers of the five new districts also has been announced.

GEORGE KALL, Denver, has been named district manager of the Colorado District. GRANT DOUGALL, Boise, was appointed district manager of the Idaho District; D. O. LAMB, Salt Lake City, for the Utah District; PAUL R. MCCLURE, Great Falls, for the Montana District; and HARLAN J. NISSEN, Los Angeles, for the California District.

A native of Denver and a graduate of Stanford University, Cannon joined Beatrice Foods as an auditor in 1940, immediately after earning his M.B.A. degree at the Harvard Graduate School of Business Administration. He was named manager of the Denver plant in 1945, Colorado district manager in 1947, a vice-president of the company in 1948, and a director in 1950.

Kall began as a supply clerk for Fairmont Foods, Moorhead, Minn., in 1927. He progressed steadily through the other office jobs to service in the field as retail route salesman, retail route supervisor, wholesale route supervisor, and then sales manager. He joined Beatrice Foods as an ice cream salesman in Denver in 1946 and then became ice cream sales manager.

A veteran of 28 years' service with Beatrice Foods in Montana, McClure started with the company at the Yellowstone Creamery in Billings in 1929. He was promoted to manager of the Beatrice plant at Helena the next year and to manager of the plant in Great Falls in 1933. In 1955, he was appointed district manager for Montana.

Nissen has been with the Terminal Refrigerating Company of Los Angeles (a Beatrice affiliate) for 39 years. He started in the office and subsequently became assistant manager, then vice-president and general manager. He was appointed division manager for all Beatrice Foods plants in California in June, 1955.

Dougall formerly was a vice-president and director of Creameries of America, now affiliated with Beatrice Foods. A native of Salt Lake City, he started work at the age of 15 and in 1941 was named manager of the Idaho Division of Creameries of America, a position he retained when the company joined Beatrice Foods.

A veteran of 34 years' experience with Beatrice Foods, Creameries of America, and Crescent Creamery, Lamb is a graduate of the Univ. of California, Berkeley, where he majored in dairy manufacturing. He was appointed general manager of the Utah Division of Creameries of America in 1942 and subsequently became a vice-president and a director. When that company merged with Beatrice, he became division manager of the Utah Division.

Univ. of Connecticut News

LEONARD R. DOWD, associate professor of dairy manufacturing, Univ. of Connecticut, Storrs, has gone on a sabbatical leave to visit dairy plants in several states, study at Ohio State Univ., and attend the A.D.S.A. Annual Meeting and other meetings. He will return to his duties August 15.

Publication on Dairy Cattle Nutrition

Publication 464, Number III, entitled "Nutrient Requirements of Domestic Animals," revised 1956, has been issued by the National Academy of Sciences–National Research Council. This is a report of the Committee on Animal Nutrition, prepared by the Subcommittee on Dairy Cattle Nutrition. Members of the Subcommittee are J. K. LOOSLI, chairman, R. B. BECKER, P. H. PHILLIPS, J. C. SHAW, and C. F. HUFFMAN, president of A.D.S.A.

The bulletin is a model of dairy cattle nutritional research, composed of sections on nutritional requirements, validation of requirements, symptoms of nutritional deficiences, composition of feeds, examples of adequate rations, and list of references.

Virginia Tech News

A highly successful Dairy Science Career Day was held on the V.P.I. campus on April 10. Seventy-five high school students and 24 representatives of the Virginia dairy industry toured the campus, inspected the dairy science herds, farm, processing plant, and laboratories for the morning portion of the program. The afternoon session dealt with a series of talks by Dean of Agriculture L. B. DIETRICK; Dr. FULLER, Director of Admissions; J. E. FLORA, a

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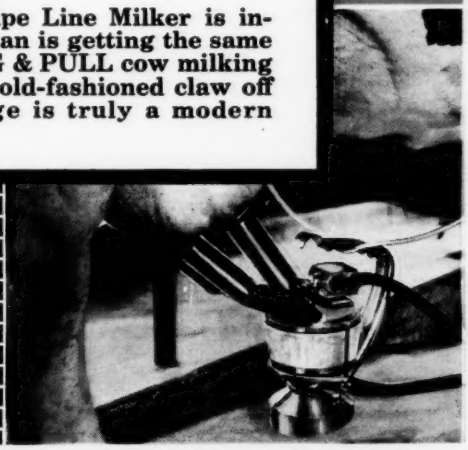

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dairy science alumnus; and four dairy science students, OSCAR KENNEDY, FRED SCOTT, PAUL KIPPS, and DAN BIRD. G. C. GRAF, head of the Dept. of Dairy Science, presided during the afternoon session.

The Career Day was sponsored by the Virginia Dairy Products Association, the Virginia State Dairymen's Association, the Virginia Chapter of A.D.S.A., and the Dept. of Dairy Science. It is hoped to make this an annual affair, since the need for informing high school students about the opportunities in the Dairy Industry is highly desirable, and more college-trained men and women are needed urgently by the Virginia dairy industry.

The Virginia Dairy Fieldmen's Association held its annual meeting in the Dairy Science Building on April 4 and 5. Over 85 fieldmen attended the two-day session, which included talks by Dr. GRAF, Dr. ELLMORE, and Dr. BALDWIN of the dairy science staff, Prof. LOOPE of the Agricultural Economics Dept., Prof. KITE of the Agricultural Engineering Dept., and a number of fieldmen and commercial dairy supply company representatives.

An artificial insemination technicians' training course was held April 22 to May 4. This course was sponsored by the Virginia Agricultural Extension Service and the Depts. of Dairy Science and Animal Pathology. V. L. BALDWIN, Dept. of Dairy Science, was in charge of the course.

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The 24th annual edition of the *Milky Way* has been published by the Virginia Polytechnic Institute, Blacksburg, Chapter of the American Dairy Science Association.

Written and edited exclusively by dairy students at V.P.I., the 68-page printed publication gives a word and picture account of the club's activities during 1956.

Whey Test for Brucellosis Tried in California

"Brucellosis, formerly the major scourge of the cattle industry, is on its way toward eradication," predicts HUGH S. CAMERON of the School of Veterinary Medicine, Univ. of California, Davis.

A pilot program to test the efficiency of an economical new whey test for brucellosis, under field conditions, is to be conducted in Marin county, Calif. "The objective of this program," said Dr. Cameron, "is to show that an area can become modified certified brucellosis-free through the use of the whey test instead of the blood test. If whey testing is approved, California could well become a modified certified brucellosis-free area within a period of two years."

"It will probably take at least 10 months to complete the necessary testing of all the cows in Marin county. If the whey test is successful, Marin county will be certified and the test will undoubtedly receive official approval of the U. S. Department of Agriculture," Dr. Cameron also said.

Dr. Cameron, who adapted the whey test, has been urging its adoption since conclusion of his own tests on more than 20,000 cows. These tests showed that the whey test is at least as efficient as the blood test and is much easier to conduct. For physical reasons, the blood test has proved impractical in California.

The pilot program is a cooperative project between the Univ. of California's School of Veterinary Medicine, the California Dept. of Agriculture, and the USDA.

Ohio State News

More than 50 people attended the sixth annual Milk Sanitarians' Short Course, April 1-5, sponsored cooperatively by the Ohio Dept. of Health, the Ohio Dept. of Agriculture, and the Dept. of Dairy Technology. The main topics discussed were: Uniformity in Interpretation of Milk Ordinances and Codes, Inspection Techniques, Recent Advances in Farm and Plant Equipment.

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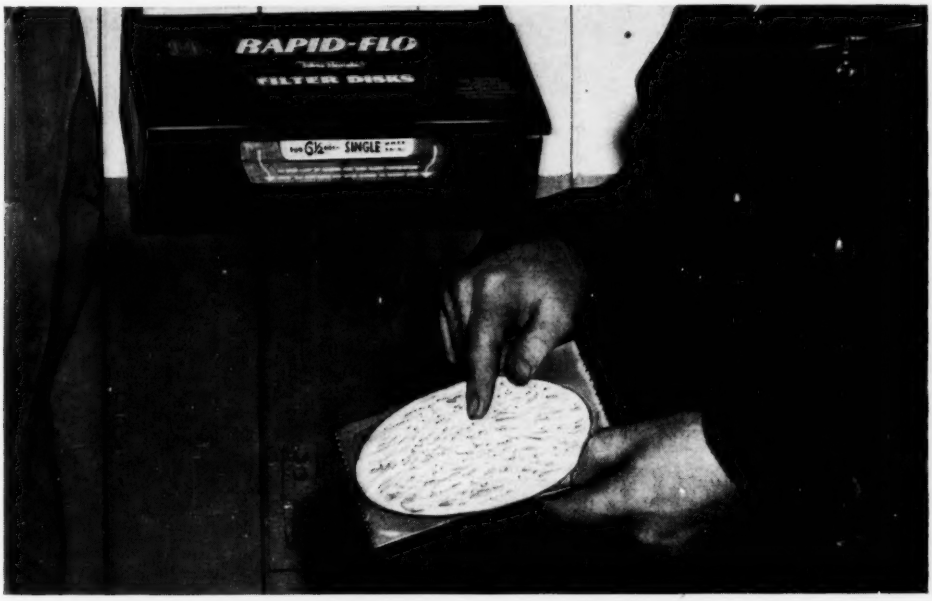

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The visiting faculty included: W. L. MALLMAN, Michigan State Univ., East Lansing; T. A. BATSCHE, Cincinnati Health Dept.; FRED DONALDSON, Canton Health Dept.; L. C. BURKEY, Creamery Package Co.; C. M. CROSBY, DeLaval Separator Co.; R B. DOUGLAS, Beatrice Foods Co.; F. HOLMAN, Babson Bros., LEROY STEVENS, Farmers' Feeder Co., and GLENN WAGNER, Northwestern Coop. Sales Assn.

Over 200 high school students, counselors, and industry representatives attended the fourth annual Dairy Technology Career Day at Ohio State Univ. on April 13. This event constituted the climax of the year's high school counseling and recruitment program, conducted cooperatively by the organized dairy industry groups of Ohio and the Dept. of Dairy Technology.

Applicants for the 20 scholarships offered to prospective freshmen in dairy technology were interviewed by industry and university representatives, April 27 and May 4, at Toledo, Cleveland, Dayton, and Columbus. The recipients will be selected on the basis of the interview, their high school record, and written examinations in the fields of science, mathematics, and English.

DALE A. SEIBERLING has returned to Ohio State after completing a 10-week assignment with the Technical Cooperation Mission, New Delhi, India, During his stay in India, he developed plans and equipment specifications for nine plants to be constructed during the Government of India's second five-year plan. Included were cooperative milk plants of 1,200 and 2,000 gallons per day, a rural creamery, a milk drying plant, two training plants for Agricultural Colleges, the training plant for the Karnal National Dairy Research Institute, and two large combination fluid milk-butter-ice cream plants for Calcutta and New Delhi, having capacities of 500,000 and 700,000 pounds daily, respectively.

A new feature of the Indian dairy industry will be the utilization of rural collection and shipping centers to receive milk from the villages, with tanker delivery to the processing plants.

R. G. DREYFUSS, Assistant Manager of Research, Thatcher Glass Manufacturing Co., Inc., Elmyra, N. Y., spoke on "Nu Glass Process" at the April meetings of the Maumee Valley, Northeastern Ohio, Cincinnati, and Central Ohio Dairy Technology Societies.

The Ohio Dairy Products Association held its third annual Accounting and Office Management Conference in Columbus, April 25. "Fleet Costs," "Automation of the Office," and "Bulk Tank Pick-Up and Accounting Problems" were the main topics discussed.

Speakers for the day included JUDY BRONDES, Findlay, O.; W. E. L. YOUNG, Columbus; E. J. LUTES, Marion, Ind., and JAMES HEALY, Columbus.

London Society Awards Gold Medal

The London, England, Society of Dairy Technology has announced the award of its Gold Medal to H. D. KAY, C.B.E., D.Sc., F.R.S., director of England's National Institute for Research in Dairying. The award has been made in recognition of Prof. Kay's distinguished service to the dairy industry in England throughout the past 25 years, and to the Society, of which he was Founder-President, since its formation in 1943. The Gold Medal will be presented to Prof. Kay at a special meeting of the Society in London on October 21, 1957.

Michigan State News

N. P. RALSTON, head of the Michigan State Univ. Dairy Dept., has completed his service as chairman of Governor Williams' Milk Marketing Advisory Board. This committee, composed of representatives of farmers, dairy plant operators, labor organizations, food dealers, and consumers, was asked to report on milk produc-

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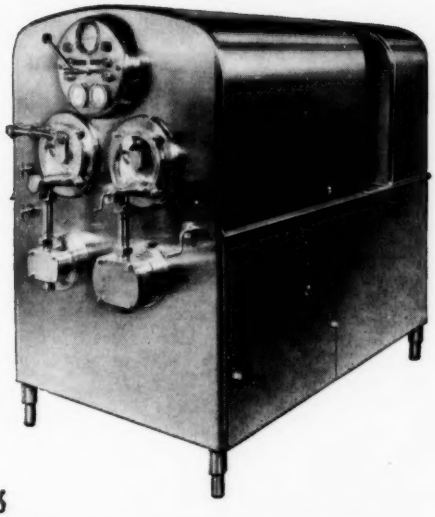

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tion economics, dairy plant operation economics, distribution costs, dairy products and the family food dollar, and state milk legislation. Their findings were made known in a 34-page circular.

Because of producer unrest, the reactions of group members to the need for milk control laws proved to be the more interesting of the subjects studied. For legislation on milk control in Michigan, 13 voted "yes," three voted "no," five abstained from voting, and nine were absent when the vote was taken.

Over 100 persons attended the Michigan Dairy Memorial and Scholarship Foundation day meeting and luncheon held April 4 at the Animal Industries Building. Twelve dairy leaders or their representatives were presented plaques in recognition of the scholarships made available in their honor. Organized within the last few years, the fund already exceeds $30,000. HAROLD OTZMAN of Foremost Dairies, Detroit, presided and CLARK BRODY accepted the fund on behalf of the State Board of Agriculture. Among those honored were TURNER H. BROUGHTON, JAMES G. HAYS, SAMUEL T. ANGOTT, G. DEWEY MCDONALD, WILLIAM A. MCDONALD, BERNARD F. BEACH, G. S. COFFMAN, PETER JOPPE, and the late A. C. BALTZER, JOHN J. MCDONALD, J. NEAL LAMOREAUX, and CLARENCE A. BRODY. The large bronze scroll carrying these names reads, "In grateful recognition for leadership and distinguished service to the Michigan dairy industry."

The Dairy Dept. faculty has been busy with several conferences sponsored directly by the department or with cooperating departments. These include: Feed Utilization by Dairy Cows, scheduled for May 23-25; and number among outside speakers C. B. BENDER, Sperry Rand Corporation; KEITH A. HUSTON, Kansas State; MAX KLEIBER, Univ. of California; R. O. OLSON, L. A. MOORE, G. E. FRICK, and R. L. MIGHALL of the USDA; A. G. NORMAN, president of the American Society of Agronomy; L. S. ROBERTSON, Purdue Univ.; HAROLD JENSEN of Univ. of Kentucky; N. F. COLOVOS, Univ. of New Hampshire; TOM REED and J. K. LOOSLI, Cornell; EARL O. HEADY, Iowa State College; and H. L. LUCAS, JR. of North Carolina State College.

The Dairy Fieldmen's Conference, April 16-17, was held in conjunction with the Michigan Association of Sanitarians, and brought in as an out-of-state speaker, LEONARD DOWD, Univ. of Connecticut, Storrs.

The Agricultural Marketing Clinic was held April 18. One section was devoted to marketing dairy products.

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Carnation Elects New Directors

Stockholders of Carnation Company, Los Angeles, Calif., at their annual meeting voted to increase the number of directors from 11 to 15 and elected four men to fill the new posts.

Elected were SHANNON CRANDALL, JR., president of California Hardware Company; ROBERT C. EVANS, a Carnation vice-president; FRED W. HOOVER, president of Albers Milling Company, a Carnation subsidiary; WILLIAM D. DOBSON, executive vice-president of General Milk Company, Carnation's foreign affiliate.

Training Program for Steroid Biochemistry

Applications are now being accepted for the second course in the Training Program for Steroid Biochemistry to start Oct. 1, 1957. The program is conducted through the cooperative effort of the Worcester Foundation for Experimental Biology; the Dept. of Chemistry, Clark Univ., Worcester, Mass.; and the Dept. of Biochemistry, College of Medicine, Univ. of Utah, Salt Lake City.

The training program is sponsored by the National Cancer Institute of the National Institutes of Health, to provide specialized training for individuals interested in steroid investigation. Two groups of candidates will be selected and will receive stipends during the period of training.

Post-doctoral candidates having an M.D. or Ph.D. degree will receive $5,000 for one year starting Oct. 1, 1957. The training will consist of laboratory and lectures covering theoretical and practical aspects of steroid research, and an opportunity to engage in a research problem under an established investigator.

Candidates having a B.S., M.S., or equivalent degree will receive $1,500 for a six-months training period, Oct. 1, 1957 through March, 1958. The pre-doctoral program is intended to provide competency in the analysis of steroid compounds for research and clinical laboratories.

The closing date for applications will be August 15, 1957. Requests for applications should be made to Dr. KRISTEN EIK-NES, Dept. of Biochemistry, College of Medicine, Univ. of Utah, Salt Lake City, Utah, or to Dr. FRANK UNGAR, Dept. of Chemistry, Clark Univ., Worcester, Mass.

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Sixteen Nations Share in U.S. Dairy Imports

Sixteen foreign countries shipped dairy products into the United States under quota authorizations set by presidential proclamation for the fiscal year ending June 30, 1957, according to a report from the USDA.

Some 20,700,000 pounds of cheese imports were authorized, including Cheddar, Blue-Mold, Edam, and Gouda, and Italian types. The major shipments come from Argentina, Canada, Denmark, Italy, the Netherlands, and New Zealand. The butter quota of 707,000 pounds was filled to within a few pounds, mostly by shipments from New Zealand and Denmark. Australia was the major shipper of nonfat dry milk and, with Canada, filled the quota of 1,807,000 pounds. The import quotas are established under Sec. 22 of the Agricultural Adjustment Act.

Agriculture Painfully Lacking in Research, According to U.S. Commission Report

Agriculture is losing by default in the contest for consumer acceptance, according to the U. S. Commission on Increased Industrial Use of Agricultural Products. The reason is that agriculture is being researched right out of its own natural domains. It is losing out to synthetics in fields that a few years ago were regarded as natural monopolies of certain crops.

Time was, reminded the Commission in its recent interim report to Congress, when the only fibres used were from cotton, wool, flax, hemp, and silk. Soap was made from agricultural oils and fats. Adhesives came from starch, glue, and plant gum. Shoes and traveling bags were made almost wholly from leather. Paints were made from vegetable oils; alcohol from molasses and corn.

But no more! The chemical and petroleum industries foresaw the possibilities of research. It is estimated by the Commission that they invest from four to seven per cent of their annual gross sales in research; that all manufacturing industry invests about three per cent. American industries, it reports, are apparently investing currently in research and development at least $3 billion annually.

"In painful contrast, total expenditures for agricultural research are estimated not to exceed $375 million, of which approximately $190 million comes from federal and state governments." The agricultural total is only slightly over one-half of one per cent of farmers' total sales—and nearly all of their research is devoted to improving and increasing production.

"Industry will continue to explore the unknown for new products and new uses for old products," concludes the Commission. "Nothing is plainer in the economic pattern of today than that agriculture must compete in the areas of basic and applied scientific research."

Charest Heads Borden of Canada

JEAN J. CHAREST has been elected president of The Borden Company, Limited, a Canadian subsidiary of the Borden Company, with headquarters in Toronto, Ont. Mr. Charest succeeds W. FRANK JONES, who has retired after nearly 50 years' service in the dairy industry. Mr. Jones is remaining with the company in an advisory capacity as a member of the board.

Mr. Charest, former executive vice-president of The Borden Company, Limited, has been associated with the dairy industry since 1921, when he joined J. J. Joubert Limited of Montreal, which became associated with Borden's in 1930. In 1934 he became Joubert's vice-president, and was elected president in 1942. In 1948, Mr. Charest was named a vice-president of The Borden Company, Limited and moved from Montreal to Toronto. He became executive vice-president in 1956.

A native of England, Mr. Jones studied at Ontario Agricultural College and held positions with the Ontario and Canadian Departments of Agriculture.

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John Brandt Scholarship Offered

The JOHN BRANDT Memorial Foundation will again offer a scholarship for graduate study in Dairy Industry, Dairy Bacteriology, or Dairy Husbandry, at the Univ. of Minnesota, St. Paul.

The scholarship furnishes $2,400 and tuition, and will be awarded for a one-year period beginning July 1 or Oct. 1, 1957. It may be renewed for an additional year if the recipient's progress warrants it.

Candidates for the scholarship should be either persons who wish to begin graduate study for the first time, or students who have completed the requirements for the M.S. degree and who plan to continue their studies toward the Ph.D. degree. For application forms, write to the Dept. of Dairy Husbandry, Institute of Agriculture, Univ. of Minnesota, St. Paul 1.

Gullickson Serves in India

THOR W. GULLICKSON, who retired last year from the Dairy Husbandry staff of the Univ. of Minnesota after 36 years' service, is now working in India as a member of the U. S. Technical Cooperation Mission. Dr. Gullickson is serving in New Delhi as an animal nutrition advisor.

Ralston Purina Fellowships Awarded

Nine recipients of Ralston Purina Research Fellowships have been announced by the Awards Committee, which met recently in St. Louis to make their selections. They are as follows:

Poultry Husbandry—DON DE STEIGUER, Oklahoma A & M, Stillwater; JOHN V. SPENCER, Purdue Univ., West Lafayette, Ind.; WILLIAM R. FEATHERSTON, Univ. of Arkansas, Fayetteville.

Dairy Husbandry—JOHN R. SUTTIE, Univ. of Wisconsin, Madison; LLOYD E. CHRISTIE, Cornell Univ., Ithaca, N.Y.; WERNER JACOBSEN, Rutgers Univ., New Brunswick, N. J.

Animal Husbandry—EARLE E. LISTER, Cornell Univ.; J. ELTON ZIMMERMAN, Oklahoma A & M; JOHN E. MOORE, Ohio State Univ., Columbus.

Alternates are RAINO K. LANSON, Orono, Me.; CORLETTE C. CALVERT, Lafayette, Ind.; MARION M. ANDERSON, Columbia, Mo.; ALFRED A. CROIX, College Station, Tex.; JACK E. MCCROSKEY, Maysville, Okla.; THOMAS W. WHITE, Lexington, Ky.; JAMES C. HRUSKA, Neenah, Wis.

This is the ninth year of the program and each award is for $1,620 on an annual basis. The selection committee is composed of representatives of the Poultry Science Association, the American Dairy Science Association, the American Society of Animal Production, the American Veterinary Medical Association, and the American Association of Land Grant Colleges and State Universities.

Colorado Symposium Follows A.D.S.A. Meeting

Colorado A & M, Fort Collins, will be host to the third annual Symposium on Reproduction and Infertility July 1-3, 1957. The program will be sponsored jointly by the College of Veterinary Medicine and the Agricultural Experiment Station.

As has been the custom with the first two symposia, the conference is arranged to immediately follow the Annual Meeting of the American Dairy Science Association, which will be held this year June 26-29 at Oklahoma A & M College, Stillwater.

Speakers will discuss the latest research in the following areas: Diseases of Reproduction in Male and Female: Ovarian Physiology: Steroid Physiology and Therapy, and Artificial Insemination.

Included among the speakers are W. E. MORGAN, president of Colorado A & M; S. S. WHEELER, director of the Colorado Agricultural Experiment Station; RUE JENSEN, dean of the Colorado A & M School of Veterinary Medicine; V. A. MILLER, Dept. of Bacteriology, Colorado A & M; KENNETH MCENTEE, Dept. of Pathology and Bacteriology, Cornell Univ., Ithaca; D. G. MCKERCHER, Dept. of Microbiology, Univ. of California, Davis; BLAINE MCGOWAN, Veterinary Clinic, Univ. of California; WAYNE A. ANDERSON and C. L. DAVIS, Animal Disease and Parasite Research Div., USDA; MALDOLM S. TRUEBLOOD, Dept. of Veterinary Medicine, Univ. of Wyoming, Laramie; WILLIAM HANSEL, Dept. of Animal Husbandry, Cornell Univ.; RICHARD J. BLANDAU, Dept. of Anatomy, Univ. of Washington, Seattle; R. O. BERRY, Dept. of Animal Husbandry, Texas A & M, College Station; A. V. NALBANDOV, Dept. of Animal Science, Univ. of Illinois, Urbana; R. C. FOLEY and J. S. GREENSTEIN, Dairy and Animal Science Dept., Univ. of Massachusetts, Amherst; JAMES A. WILLIAMS, CLIFFORD W. DUNCAN, and DONALD H. MCWADE, Dept. of Veterinary Pathology, Michigan State Univ., East Lansing; L. T. SAMUELS, Dept. of Biological Chemistry, Univ. of Utah, Salt Lake City; WILLIAM G. ROBERTSON, Veterinary Medicine Dept., Schering Corporation, Bloomfield, N. J.; E. C. REIFENSTEIN, JR., associate medical director, E. R. Squibb & Sons, N. Y.; JOEL BITMAN, T. R. WRENN, and J. F. SYKES, Dairy Husbandry Research Branch, USDA; H. E. BREDECK and D. T. MAYER, Dept. of Agricultural Chemistry, Univ. of Missouri, Columbia; WRIGHT H. LANGHAM, chief of biomedical research, Univ. of California, Los Alamos Sci-

entific Laboratory, Los Alamos, N.M.; W. E. PETERSEN, JAMES R. NICHOLS, EDWARD GRAHAM, and JOHN DONKER, Dept. of Dairy Husbandry, Univ. of Minnesota, St. Paul; F. X. GASSNER, Dept. of Chemistry, Colorado A & M; JOHN E. NELLOR, Dept. of Physiology and Pharmacology, Michigan State Univ.; S. W. MEAD, Dept. of Animal Husbandry, Univ. of California; PHILLIP M. HINZE, Carnation Milk Farms, Carnation, Wash.; H. J. HILL, Dept. of Clinics and Surgery, Colorado A & M; HENRYK NOWAKOWSKI, Medical Univ. Clinic, Hamburg-Eppendorf, Germany; R. J. FLIPSE, Dairy Cattle Breeding Research Center, Pennsylvania State Univ., University Park; RICHARD KELLOGG, Central Ohio Breeding Association, Columbus; M. H. EHLERS, Dept. of Dairy Science, State College of Washington, Pullman; and CHARLES P. STROBLE, Dept. of Animal Production, Univ. of Wyoming, Laramie.

Judkins' Visit Florida

Mr. and Mrs. H. F. Judkins returned home in April from a vacation in Florida. In addition to resting, golfing, and swimming, they visted friends, including two Past Presidents and Honorary members of the A.D.S.A., Dr. H. B. ELLENBERGER, 1317 Eastin Street, and Prof. J. H. FRANDSEN, 1510 Amelia Avenue, Orlando. Dr. Ellenberger is a fisherman of some repute and raises beautiful roses. The Frandsens recently purchased a home in Orlando, but plan to spend their summers in Amherst, Mass.

Where to Send It

Throughout the year many members and subscribers may have occasion to correspond with the Association about subscriptions to *The Journal of Dairy Science.*

So that all inquiries may be given prompt attention and quick action, the Association requests that you make a note of this brief outline of "where to send it."

Membership correspondence (including material relative to members' subscriptions to the Journal) — The American Dairy Science Association, c/o H. F. JUDKINS, Secretary-Treasurer, 32 Ridgeway Circle, White Plains, N.Y.

Nonmember subscription correspondence — The Journal of Dairy Science, c/o The Garrard Press, 510-522 North Hickory Street, Champaign, Illinois.

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LETTER TO THE EDITOR

In looking over recent issues of the Journal, I have been impressed by the wide diversity in the summaries of individual papers. Some authors write a summary which is, in a manner of speaking, merely the "final paragraph of the paper." Such a summary is meaningless unless one reads the entire paper. Other authors present a summary which is more in the nature of an abstract, in that it presents a brief description of what was done, how it was done, and what was found. I believe that this situation indicates the need for an analysis of the purpose and function of the summary and the establishment of an editorial policy specifying its form and content.

Two principal functions of the summary are readily discernible. One is to present the conclusions which the author derives from the experimental work. This function can best be handled in the discussion, with informal or formal "conclusions," as the author desires. The other function is to orient the reader with regard to the material covered by the paper. I am sure that many readers consider the summary primarily from the standpoint of orientation and turn to it before actually reading the paper. Personally, I think that this orienting function would best be performed by an informative abstract prefacing the paper. If the reader is only casually interested in the subject he need go no further than the abstract. If, on the other hand, he is deeply interested in the subject, a preliminary perusal of the abstract will improve the efficiency with which he reads the paper. Obviously, the "editorial interpretations" now prefacing each paper in the Journal are intended as "pre-reading" orientation. However, these interpretations are too brief and too general to perform this function adequately. A well-organized and factual abstract would be much more suitable for the purpose.

I would like to propose that both the present "editorial interpretation" and the terminal summary be abolished and be replaced by an abstract placed at the beginning of the paper. This abstract should be prepared by the author and carefully edited. Serious effort should be devoted to making it complete, factual, and interpretive. I think that this change would greatly improve our Journal.

ROBERT JENNESS
Department of Agricultural Biochemistry
University of Minnesota
St. Paul 1, Minnesota

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PROGRAM

FIFTY-SECOND ANNUAL MEETING

of the

AMERICAN DAIRY SCIENCE ASSOCIATION

OKLAHOMA A & M COLLEGE

STILLWATER, OKLAHOMA

June 26-29, 1957

Program Committee

N. P. RALSTON, Michigan, *Chairman*
I. A. GOULD, Ohio, *Ex-Officio*
J. D. BURKE, New York
S. W. MEAD, California
C. L. NORTON, Oklahoma
H. L. TEMPLETON, Nebraska

GENERAL PROGRAM

Tuesday, June 25

9:00 A.M. **Meeting of the Executive Board,** *Student Union Council Room*

Wednesday, June 26

9:00 A.M. **Meeting of the Executive Board,** *Student Union Council Room*

10:00 A.M. **Registration,** *Student Union (Second Floor)*

5:30 P.M. **Past Presidents' Dinner,** *President's Dining-Room*

8:00 P.M. **Informal Reception,** *Student Union Ballroom*

Thursday, June 27

8:00 A.M. **Registration,** *Student Union (Second Floor)*

9:30 A.M. OPENING SESSION, *Student Union Ballroom*
DR. C. L. NORTON, *Head, Department of Dairying, Oklahoma A & M College, presiding.*

Prelude

National Anthem

PROFESSOR L. N. PERKINS, *Department of Music, Oklahoma A & M College.*

Invocation

REVEREND S. M. MADDOX, *Pastor, First Baptist Church, Stillwater.*

Welcome

DR. L. E. HAWKINS, *Director, Oklahoma Agricultural Experiment Station.*

Presidential Address: The Road Ahead

DR. C. F. HUFFMAN, *Michigan State University.*

Address: The Role of Agriculture in World Diplomacy

DR. R. T. KLEMME, *Director, Department of Commerce and Industry, State Capitol, Oklahoma City.*

Postlude

MANUFACTURING SECTION

All meetings in Student Union

Thursday, June 27

2:00–4:00 P.M.

SECTION A. **Lactose Symposium**
Student Union Varsity Room

2:00–4:00 P.M.

JOINT WITH EXTENSION AND PRODUCTION SECTIONS

Selected Topic: Teaching Methods
(SEE EXTENSION SECTION)
Student Union Basement, Room 2

4:10 P.M.

MANUFACTURING SECTION BUSINESS MEETING
Student Union Varsity Room

Friday, June 28

8:45–11:15 A.M.

SECTION A. **Milk Proteins**
Student Union Varsity Room

9:00–11:00 A.M.

EDUCATION COMMITTEE (JOINT MANUFACTURING, PRODUCTION, AND EXTENSION SECTIONS)
Student Union Pioneer Room

Selected Topic: Curriculum and Course Organization for Dairy Science
E. L. JACK, *Chairman*
Dairy Production. H. H. COLE, *University of California*
Dairy Manufacturing. P. H. TRACY, *University of Illinois*

11:15 A.M.–12:15 P.M.

MANUFACTURING SECTION BUSINESS MEETING
Student Union Varsity Room

2:00–5:00 P.M.

SECTION A. **Dairy Chemistry**
Student Union Varsity Room

2:00–5:00 P.M.

SECTION B. **Dairy Bacteriology**
Student Union Pioneer Room

5:00 P.M.

JOINT WITH PRODUCTION AND EXTENSION SECTIONS
Selected Topic: Solids-Not-Fat and Its Constituents
(SEE PRODUCTION SECTION)
Classroom Building Auditorium

Saturday, June 29

8:30–10:15 A.M.

SECTION A. **Dry Milk**
Student Union Varsity Room

8:30–10:15 A.M.

SECTION B. **Cheese**
Student Union Pioneer Room

9:00–10:15 A.M.

STUDENT AFFILIATES AND FACULTY ADVISORS' MEETING
The Student Affiliate Program for 1957-58
W.L. SLATTER, *Chairman*
Student Union Corral Room

10:30 A.M.–12:30 P.M.

GENERAL BUSINESS SESSION OF THE ASSOCIATION
C. F. HUFFMAN, *Chairman*
Student Union Ballroom

2:00–5:00 P.M.

JOINT WITH PRODUCTION AND EXTENSION SECTIONS

Symposium: Legal and Regulatory Aspects of Dairy Products Manufacture

B. H. WEBB, *Chairman*

Student Union Ballroom

2:00–4:15 P.M.

SECTION B. **Dairy Products**

Student Union Varsity Room

PRODUCTION SECTION

All meetings in Classroom Building

Thursday, June 27

1:45–5:30 P.M.

SECTION A. **General Nutrition**

Classroom Building Auditorium

1:45–5:30 P.M.

SECTION B. **Physiology**

Classroom Building, Room 112

2:00–4:00 P.M.

JOINT WITH EXTENSION AND MANUFACTURING SECTIONS

Selected Topic: Teaching Methods

(SEE EXTENSION SECTION)

Student Union Basement, Room 2

Friday, June 28

8:00–11:15 A.M.

SECTION A. **Silage and Pasture**

Classroom Building Auditorium

SECTION B. **Artificial Insemination**

Classroom Building, Room 112

9:00–11:00 A.M.

EDUCATION COMMITTEE (JOINT MANUFACTURING, PRODUCTION, AND EXTENSION SECTIONS)

Selected Topic: Curriculum and Course Organization for Dairy Science

(SEE MANUFACTURING SECTION)

Student Union Pioneer Room

11:30 A.M.–12:30 P.M.

PRODUCTION SECTION BUSINESS MEETING
Classroom Building Auditorium

1:30–4:00 P.M.

SECTION A. **Milk Secretion**
Classroom Building Auditorium
SECTION B. **Calf Nutrition**
Classroom Building, Room 112

4:00–5:00 P.M.

JOINT WITH EXTENSION SECTION
Joint Committee Reports
S. W. MEAD, *Chairman*
Classroom Building Auditorium

5:00 P.M.

JOINT WITH EXTENSION AND MANUFACTURING SECTIONS
Selected Topic: Solids-Not-Fat and Its Constituents
S. N. GAUNT, *Chairman*
Classroom Building, Room 112
JOINT WITH EXTENSION SECTION
MEETING OF SUPERINTENDENTS OF OFFICIAL TESTING
(SEE EXTENSION SECTION)
Classroom Building Auditorium

Saturday, June 29

8:00–10:15 A.M.

SECTION A. **Reproduction**
Classroom Building Auditorium
SECTION B. **Genetics**
Classroom Building, Room 112

9:00–10:15 A.M.

STUDENT AFFILIATES AND FACULTY ADVISORS' MEETING
(SEE MANUFACTURING SECTION)
Student Union Corral Room

10:30 A.M.–12:30 P.M.

GENERAL BUSINESS SESSION OF THE ASSOCIATION
C. F. HUFFMAN, *Chairman*
Student Union Ballroom

2:00–5:00 P.M.

JOINT WITH MANUFACTURING AND EXTENSION SECTIONS
Symposium: Legal and Regulatory Aspects of Dairy Products Manufacture
(SEE MANUFACTURING SECTION)
Student Union Ballroom

2:00–5:30 P.M.

SECTION A. **Rumen Physiology**
Classroom Building Auditorium

EXTENSION SECTION

All Meetings in Student Union Basement, Rooms 1, 2, 3

Thursday, June 27

2:00–4:00 P.M.

JOINT WITH MANUFACTURING AND PRODUCTION SECTIONS
Selected Topic: Teaching Methods
J. D. BURKE, *Chairman*
Student Union Basement, Room 2

Visuals and You. D. T. SCHILD, *Division of Information Programs, USDA.*

Cow Colleges: A New Approach to Winter Meetings. R. W. SPALDING, *Cornell University.*

A Feeding School in Every County on the Same Day. J. G. CASH, *University of Illinois*

4:30–5:00 P.M.

PRESENTATION AND DISCUSSION OF EXHIBITS
L. H. STINNETT, *Chairman*
Student Union Basement, Rooms 1, 2, 3

Friday, June 28

9:00–11:00 A.M.

EDUCATION COMMITTEE (JOINT MANUFACTURING, PRODUCTION, AND EXTENSION SECTIONS)
Selected Topic: Curriculum and Course Organization for Dairy Science
(SEE MANUFACTURING SECTION)
Student Union Pioneer Room

9:00–11:15 A.M.

Milk Records and Herd Improvement
Student Union Basement, Room 2

11:15 A.M.–12:30 P.M.

EXTENSION SECTION BUSINESS MEETING
Student Union Basement, Room 2

1:30–4:00 P.M.

JOINT WITH PRODUCTION AND MANUFACTURING SECTIONS
(SEE PRODUCTION AND MANUFACTURING SECTIONS A AND B)

4:00–5:00 P.M.

JOINT WITH PRODUCTION SECTION

Joint Committee Reports

(SEE PRODUCTION SECTION)

Classroom Building Auditorium

5:00 P.M.

JOINT WITH PRODUCTION AND MANUFACTURING SECTIONS

Selected Topic: Solids-Not-Fat and Its Constituents

(SEE PRODUCTION SECTION)

Classroom Building, Room 112

5:00 P.M.

JOINT WITH PRODUCTION SECTION

MEETING OF SUPERINTENDENTS OF OFFICIAL TESTING

K. A. HUSTON, *Chairman*

Classroom Building Auditorium

Saturday, June 29

9:00–10:15 A.M.

STUDENT AFFILIATES AND FACULTY ADVISORS' MEETING

(SEE MANUFACTURING SECTION)

Student Union Corral Room

9:00–10:30 A.M.

Herd Management Phase of the 4-H Dairy Project

L. A. JOHNSON, *Chairman*

Student Union Basement, Room 2

10:30 A.M.–12:30 P.M.

GENERAL BUSINESS SESSION OF THE ASSOCIATION

C. F. HUFFMAN, *Chairman*

Student Union Ballroom

2:00–5:00 P.M.

JOINT WITH MANUFACTURING AND PRODUCTION SECTIONS

Symposium: Legal and Regulatory Aspects of Dairy Products Manufacture

(SEE MANUFACTURING SECTION)

Student Union Ballroom

2:00–5:00 P.M.

Herd Management and Breeding

Student Union Basement, Room 2

8:00 P.M.

Recognition Program

Student Union Ballroom

ENTERTAINMENT

GENERAL PROGRAM

Wednesday, June 26 — 8:00 P.M.—**Informal Reception**
Student Union Ballroom

Thursday, June 27 — 6:30 P.M.—**Barbecue and Indian Pow Wow**
Stadium

8:00 P.M.—**Square and Round Dancing**
Student Union Ballroom

Friday, June 28 — **No Entertainment**

Saturday, June 29 — 8:00 P.M.—**Recognition Program**
Student Union Ballroom

WOMEN'S PROGRAM

Thursday, June 27 — 2:00 P.M.—**Welcoming Tea**—"Magic Suitcase"
Student Union Ballroom

Friday, June 28 — All Day—**Tours**

Tulsa—Frankhoma Pottery, Visit
Luncheon, *Oak Country Club*

Ponca City—Continental Oil Company,
Visit and Luncheon

Saturday, June 29 — 10:00 A.M.—**Brunch**
Student Union, Parlors A, B, C

Saturday, June 29 — 2:30 P.M.—**Farewell Coffee**
Student Union, Chinese Lounge

CHILDREN'S PROGRAM

(See separate program)

CHILDREN 3-5 YEARS

Thursday, June 27 — 9:00-11:45 A.M.
—**Supervised Care**
Children's Center, Campus

2:00–4:30 P.M.
—**Supervised Care**
Children's Center, Campus

Friday, June 28 8:30 A.M.–4:30 P.M.
—**Supervised Care**
Children's Center, Campus

Saturday, June 29 9:00-11:45 A.M.
—**Supervised Care**
Children's Center, Campus

2:00–4:30 P.M.
—**Supervised Care**
Children's Center, Campus

CHILDREN 6-8 YEARS

Thursday, June 27 9:00 A.M.–NOON
—**Campus Hike, Games**

1:30–4:30 P.M.
—**Magic Show, Games, Movies**

Friday, June 28 8:30 A.M.–4:30 P.M.
—**Games, Tour to Pawnee, and Picnic at** *Pawnee City Lake*

Saturday, June 29 9:00 A.M.–NOON
—**Games and Crafts**

2:00–4:30 P.M.
—**Games, Music, Movies**

CHILDREN 9-11 YEARS

Thursday, June 27 9:00–10:45 A.M.
—**Campus Hike**

11:00 A.M.–NOON
—**Swimming at** *College Pool*
(Must bring suit, cap if girl, and health statement from doctor)

1:30–4:30 P.M.
—**Magic Show, Games, Movies**

Friday, June 28 8:30 A.M.–4:30 P.M.
—**Tour to Pawnee, Indian Museum, and Picnic at** *Pawnee City Lake*

Saturday, June 29 9:00–10:45 A.M.
—**Games**

11:00 A.M.–NOON

—Swimming at *College Pool*

(Must bring suit, cap if girl, and health statement from doctor)

2:00–4:30 P.M.

—Games, Music, Movies

CHILDREN 12 YEARS AND OLDER

Thursday, June 27 9:00 A.M.–NOON

—Union and Campus Tours, Mixer

1:30–4:30 P.M.

—Magic Show, Recreation

Friday, June 28 8:30 A.M.–4:30 P.M.

—Tour to Pawnee, Pawnee Bill's Home, Indian Museum, and Picnic at *Pawnee City Lake*

Saturday, June 29 11:00 A.M.–NOON

—Swimming Party, *Crystal Plunge*

2:00–4:30 P.M.

—Recreation

Baby Sitters: List available at registration desk.

Special Recreation: Swimming, Golf, and Tennis are available.

Please bring your own sports equipment.

MANUFACTURING SECTION

All Meetings in Student Union

Thursday, June 27

2:00–4:00 P.M.

SECTION A. **Lactose Symposium**

B. H. WEBB, *Chairman*

E. W. BIRD, *Moderator*

Student Union Varsity Room

Physical-chemical aspects of lactose. R. P. CHOI, *American Dry Milk Institute, Inc., Chicago, Ill.*

Commercial production of lactose. M. E. HULL, *Armour and Co., Chicago, Ill.*

Commercial utilization of lactose. ARA CALL, *Western Condensing Co., Appleton, Wis.*

Difficulties encountered as a result of lactose crystallization in concentrated dairy products. F. J. DOAN, *Pennsylvania State University.*

2:00–4:00 P.M.

JOINT WITH EXTENSION AND PRODUCTION SECTIONS
Selected Topic: Teaching Methods (SEE EXTENSION SECTION)
Student Union Basement, Room 2

4:10 P.M.

MANUFACTURING SECTION BUSINESS MEETING
F. J. BABEL, *Chairman*
Student Union Varsity Room

Friday, June 28

8:45–11:15 A.M.

SECTION A. **Milk Proteins**
B. H. WEBB, *Chairman*
Student Union Varsity Room

M1. The principal proteins of bovine milk. R. JENNESS, *University of Minnesota.*

M2. A review of the minor proteins in bovine milk. R. McL. WHITNEY, *University of Illinois.*

M3. Binding of calcium and phosphate ions to casein at pH 5 to 7.5. CHARLES A. ZITTLE, *Eastern Utilization Research Branch, USDA, Philadelphia, Pa.*

M4. Changes produced by chymotrypsin proteolysis of casein. H. A. LILLEVIK AND R. A. ANWAR, *Michigan State University.*

M5. The solubility in aqueous urea solutions of the micellular caseinates of milk and milk products subjected to various sterilizing heat treatments. H. S. HALLER AND M. J. PALLANSCH, *Eastern Utilization Research Branch, USDA, Washington, D.C.*

M6. Molecular weight determination by osmotic pressure measurements of casein in 6.6 *M* urea. H. C. NIELSEN AND H. A. LILLEVIK, *Michigan State University.*

M7. Characterization of the milk protein fraction precipitated by salt but not by acid. R. JENNESS, *University of Minnesota.*

M8. The electrophoretic pattern of undiluted skimmilk with its native protein-free milk system as a buffer. H. K. WILSON, G. K. MURTHY, AND R. McL. WHITNEY, *University of Illinois.*

9:00–11:00 A.M.

EDUCATION COMMITTEE (JOINT MANUFACTURING, PRODUCTION, AND EXTENSION SECTIONS)

Student Union Pioneer Room

Selected Topic: Curriculum and Course Organization for Dairy Science

E. L. JACK, *Chairman*

Dairy Production. H. H. COLE, *University of California*

Dairy Manufacturing. P. H. TRACY, *University of Illinois*

11:15 A.M.–12:15 P.M.

MANUFACTURING SECTION BUSINESS MEETING

F. J. BABEL, *Chairman*

Student Union Varsity Room

2:00–5:00 P.M.

SECTION A. **Dairy Chemistry**

B. H. WEBB, *Chairman*

Student Union Varsity Room

M9. Comparison of methods for the determination of butterfat, solids-not-fat and total solids in milk. C. J. COSGROVE AND J. W. COBBLE, *University of Rhode Island.*

M10. The determination of solids-not-fat in milk by means of a subsurface centrifugal hydrometer. N. S. GOLDING, *State College of Washington.*

M11. Effects of age on the viscosity of pasteurized fractions of milk. C. H. WHITNAH, W. D. RUTZ, AND T. G. ALEXANDER, *Kansas State College* and *Eastern Utilization Research Branch, USDA, Washington, D.C.*

M12. The viscosity of milk as affected by butterfat and protein content. W. D. RUTZ AND C. H. WHITNAH, *Kansas State College.*

M13. Changing the calcium content of skimmilk and the subsequent effect upon casein. M. P. THOMPSON, W. D. RUTZ, AND C. H. WHITNAH, *Kansas State College.*

M14. A study of the acid-soluble nitrogen, phosphorus, and exchangeable calcium with special reference to the enzymic phase of rennet action in milk. I. S. VERMA AND C. W. GEHRKE, *University of Missouri.*

M15. The effect of heat and pH on the ion-exchangeable calcium and magnesium in skimmilk as determined by the "resin contact time method." C. W. GEHRKE AND B. R. SMITH, *University of Missouri.*

M16. Seasonal variations in the citric acid distribution in milk. T. A. NICKERSON, *University of California.*

M17. A gas-liquid partition chromatographic method for volatile fatty acids in milk and dairy products. C. L. HANKINSON AND W. J. HARPER, *Ohio State University.*

M18. Heat stability of pure milk fat and fat-soluble vitamins. S. RAJAN AND V. N. KRUKOVSKY, *Cornell University.*

M19. The oxidative deterioration of butter oil at various temperatures with added antioxidants. G. R. GREENBANK AND H. D. WEIHE, *Eastern Utilization Research Branch, USDA, Washington, D.C.*

M20. Observations on Kartha's and Hilditch's methods for determining the glyceride structure of fats. E. G. HAMMOND AND L. R. ESHELMAN, *Iowa State College.*

M21. Microscopy of the crystalline structure of butter. J. M. DE MAN AND F. W. WOOD, *University of Alberta.*

2:00–5:00 P.M.

SECTION B. **Dairy Bacteriology**

F. J. BABEL, *Chairman*

Student Union Pioneer Room

M22. The effect of the plating medium and plate incubation on the survival of heat-treated psychrophiles. C. D. HEATHER AND C. VANDERZANT, *Texas A & M College.*

M23. The distribution of psychrophilic bacteria in commercial dairy products. W. D. SCHULTZE AND J. C. OLSON, JR., *University of Minnesota.*

M24. A rapid test for detecting antibiotic activity in milk. K. M. SHAHANI AND M. C. BADAMI, *Ohio State University.*

M25. Observations on the method of reduction of 2, 3, 5-triphenyl tetrazolium chloride by lactic acid bacteria. B. J. LISKA AND H. E. CALBERT, *University of Wisconsin.*

M26. Nucleic acid and nucleoprotein metabolism of terramycin sensitive and resistant organisms. K. M. SHAHANI, *Ohio State University.*

M27. Influence of bacteria in the development of oxidized flavor in milk. E. B. COLLINS AND W. L. DUNKLEY, *University of California.*

M28. Citrate metabolism and carbonyl compound formation by some homofermentative lactic acid bacteria. W. J. HARPER AND ROSE M. HUBER, *Ohio State University.*

M29. Changes in the bacteriophage sensitivity of lactic streptococci. E. B. COLLINS, *University of California.*

M30. Gas production by lactic cultures infected with bacteriophage. R. L. SELLARS AND F. J. BABEL, *Purdue University.*

M31. Observations on the numbers of virus-resistant mutants developing in the host-virus system of the lactic streptococci. C. C. PROUTY AND D. PARISOT, *State College of Washington.*

M32. A low-calcium medium for propagation of lactic cultures. F. J. BABEL, *Purdue University.*

5:00 P.M.

JOINT WITH PRODUCTION AND EXTENSION SECTIONS
Selected Topic: Determination of Solids-Not-Fat
(SEE PRODUCTION SECTION)
Classroom Building, Room 112

Saturday, June 29

8:30–10:15 A.M.

SECTION A. **Dry Milk**
B. H. WEBB, *Chairman*
Student Union Varsity Room

M33. Relationship of ferricyanide-reducing capacity to keeping quality of nonfat dry milk. R. B. PARKER AND J. M. MCINTIRE, *Carnation Research Laboratories, Van Nuys, Calif.*

M34. Studies on phosphatase, soluble whey protein, and quality of nonfat dry milk made by low-temperature processing. W. K. STONE, *Virginia Polytechnic Institute.*

M35. Studies on insoluble fractions isolated from reconstituted whole milk powder. J. P. JULIEN, *Quebec Dairy School*, and B. E. BAKER, *Macdonald College.*

M36. A rapid method for determining free fat in dry milks. R. H. THOMAS, LT. C. J. HOLGREN, JR., L. JOKAY, AND I. BLOCH, *QM Food and Container Institute for the Armed Forces, Chicago, Ill.*

M37. Flavor and chemical properties of nonfat dry milk. M. KEENEY AND R. BASSETTE, *University of Maryland.*

M38. A volumetric method for estimating the undenatured serum proteins in nonfat dry milk. H. A. ANDERSON, *Eastern Utilization Research Laboratory, USDA, Washington, D.C.*

M39. Procedure for determining heat treatment received by nonfat milk. R. B. PARKER, *Carnation Research Laboratories, Van Nuys, Calif.*

8:30–10:15 A.M.

SECTION B. **Cheese**
F. J. BABEL, *Chairman*
Student Union Pioneer Room

M40. Manufacture of a rindless Swiss-type cheese. D. D. DEANE, *Iowa State College.*

M41. Flavor development and biochemical patterns of rindless block and wheel-type Swiss cheese. T. KRISTOFFERSEN AND W. L. SLATTER, *Ohio State University.*

M42. Yield, composition, and flavor of Blue cheese ripened with three types of surface coatings. D. M. GRAHAM AND W. T. WILLIAMSON, *Clemson Agricultural College.*

M43. The feasibility of producing Cheddar Cheese spreads for the armed forces, employing a HTST sterilizing process. R. I. MEYER, T. H. BURTON, J. T. HUTTON, AND H. A. HOLLENDER, *QM Food and Container Institute for the Armed Forces, Chicago, Ill.*

M44. Electron microscopic observations on the structure of curd and cheese. I. I. PETERS, J. W. DIETRICH, AND P. G. HANSEN, *A & M College of Texas.*

M45. Using the spectrophotometer for measuring cheese ripening. D. G. VAKALERIS AND W. V. PRICE, *University of Wisconsin.*

M46. The organoleptic evaluation and biochemical analysis of commercial Cheddar Cheese. T. KRISTOFFERSEN AND I. A. GOULD, *Ohio State University.*

9:00–10:15 A.M.

STUDENT AFFILIATES AND FACULTY ADVISORS' MEETING

The Student Affiliate Program for 1957-58

W. L. SLATTER, *Chairman*

Student Union Corral Room

10:30 A.M.–12:30 P.M.

GENERAL BUSINESS SESSION OF THE ASSOCIATION

C. F. HUFFMAN, *Chairman*

Student Union Ballroom

2:00–5:00 P.M.

JOINT WITH PRODUCTION AND EXTENSION SECTIONS

Symposium: Legal and Regulatory Aspects of Dairy Products Manufacture

B. H. WEBB, *Chairman*

Student Union Ballroom

The U. S. Public Health Service milk sanitation activities and their relation to state and local programs. J. D. FAULKNER, *Public Health Service.*

Federal milk orders and their effect on milk procurement. E. M. NORTON, *National Milk Producers Federation.*

The effect of government regulations and control on the marketing of dairy products. R. W. BARTLETT, *University of Illinois.*

The impact of food and drug definitions and standards of identity of dairy products manufacture. K. G. WECKEL, *University of Wisconsin.*

The USDA Grade standards program. B. J. OMMODT, *Inspection and Grading Branch, Agricultural Marketing Service.*

Grading, standards, and identification of dairy products in foreign countries; their effect on United States exports and world trade. C. J. BABCOCK, *Dairy and Poultry Division, Foreign Agricultural Service.*

2:00–4:15 P.M.

SECTION B. **Dairy Products**
F. J. BABEL, *Chairman*
Student Union Varsity Room

M47. The pantothenic acid content of cheeses. I. L. HATHAWAY, *University of Nebraska.*

M48. The influence of rate and temperature of cooking on acid development in making Cheddar Cheese from pasteurized milk. L. B. BARTON, E. R. JARMAN, AND J. J. WILLINGHAM, *Texas Technological College.*

M49. Techniques for measuring physico-chemical changes in concentrated milks. R. N. GIROUX AND H. E. CALBERT, *University of Wisconsin.*

M50. The influence of the stage of lactation on rancidity in raw milk. J. C. COLMEY, B. J. DEMOTT, AND G. M. WARD, *Colorado State University.*

M51. A rapid method for determining the efficiency of homogenization of milk. D. L. GIBSON AND E. O. HERREID, *University of Illinois.*

M52. An improved staining technique for homogenized milk fat. S. J. PEARCE AND H. SEAGRAVE-SMITH, *Carnation Research Laboratories, Van Nuys, California.*

M53. Effects of ultrasonics on milk. J. L. NEWCOMER, C. W. HALL, J. R. BRUNNER, AND C. SMITH, *Michigan State University.*

M54. Method of sample preparation for basic viscosity measurement of ice cream mix. W. A. KRIENKE AND H. B. YOUNG, *University of Florida.*

M55. Specifications for the color of butter. R. R. RIEL, *Department of Agriculture, Ottawa, Canada.*

PRODUCTION SECTION

All Meetings in Classroom Building

Thursday, June 27

1:45–5:30 P.M.

SECTION A. **General Nutrition**
S. W. MEAD, *Chairman*
Classroom Building Auditorium

P1. A comparison of stored feeding, green feeding, and grazing of pasture crops. H. J. LARSEN, R. F. JOHANNES, AND G. H. TENPAS, *University of Wisconsin.*

P2. Supplementation of cottonseed hulls with an alcohol urea preparation. E. R. Morris and O. H. Horton, *University of Arkansas.*

P3. Utilization of high levels of molasses and urea-molasses by dairy heifers. W. A. King, *Clemson College.*

P4. Progress report on a study of the effect of plane of early nutrition upon reproductive and productive performance of Holstein cattle. J. T. Reid, J. K. Loosli, K. L. Turk, S. A. Asdell, and S. E. Smith, *Cornell University.*

P5. The effect of fattening dairy heifers upon their growth and lactation. E. W. Swanson, *University of Tennessee.*

P6. The effect of varying hay-grain ratios and levels of feed intake on feed utilization of dairy cows. C. A. Lassiter, C. F. Huffman, and C. W. Duncan, *Michigan State University.*

P7. The influence of stage of maturity of alfalfa hay on its feeding value for milking cows. T. H. Blosser, F. R. Murdock, A. S. Hodgson, and P. A. Putnam, *State College of Washington.*

P8. Some factors related to roughage consumption by dairy cows. R. E. Mathier, A. A. Rimm, and J. W. Bartlett, *New Jersey Agricultural Experiment Station.*

P9. The measurement of digestibility by means of a radioactive isotope. E. A. Kane, W. C. Jacobson, and P. M. Damewood, Jr., *Dairy Husbandry Research Branch. USDA, Beltsville, Maryland.*

P10. A method for holding wet feces without nitrogen loss. W. C. Jacobson, P. M. Damewood, Jr., and E. A. Kane, *Dairy Husbandry Research Branch, USDA, Beltsville, Maryland.*

P11. Effect of methods of preservation and storage of fecal samples on energy losses. W. P. Flatt, *Dairy Husbandry Research Branch, USDA, Beltsville, Maryland.*

P12. The digestion of an all-corn ration with and without urea by lactating dairy cattle. J. H. Byers, *University of Illinois.*

P13. The effects of pasture *vs.* dry lot for dairy cattle. I. L. Hathaway and P. L. Kelly, *University of Nebraska.*

P14. A preliminary comparison of the nutritive values of orchard grass, smooth bromegrass, Kentucky 31 Fescue, and Gl-43 Fescue. D. R. Jacobson, R. H. Singer, J. W. Rust, and D. M. Seath, *University of Kentucky.*

P15. Grain feeding on pasture did not pay. D. R. Dowden and D. M. Seath, *University of Kentucky.*

1:45–5:30 P.M.

SECTION B. **Physiology**
S. P. MARSHALL, *Chairman*
Classroom Building, Room 112

P16. Effect of treatment with 0,0-dimethyl 0-2,4,5-trichlorophenyl phosphorothioate on milk production. F. N. BAKER, *North Louisiana Hill Farm Experiment Station.*

P17. Breed, season, and age differences in the 48-hour uptake of I^{131} by dairy cattle. J. R. LODGE, R. C. LEWIS, AND L. D. MCGILLIARD, *Michigan State University.*

P18. Effect of estrogen and progesterone on thyroid function of dairy cattle. G. W. PIPES AND B. N. PREMACHANDRA, *University of Missouri.*

P19. Toxicity and residue studies with phosdrin insecticide in dairy cattle. J. B. KNAAK, J. E. CASIDA, P. E. GATTERDAM, R. D. LANCE, AND R. P. NIEDERMEIER, *University of Wisconsin.*

P20. Calcium mobilization studies using a chelating agent. V. F. COLENBRANDER AND V. R. SMITH, *University of Wisconsin.*

P21. Changes in bovine serum mineral levels associated with calcium gluconate administration. K. A. KENDALL AND K. E. HARSHBARGER, *University of Illinois.*

P22. A test of the effectiveness of certain irradiated dietary supplements in preventing milk fever. K. E. HARSHBARGER AND K. A. KENDALL, *University of Illinois.*

P23. Phenolsulfonaphthalein fractional clearance in dairy cattle as a measure of renal function. J. P. MIXNER AND R. R. ANDERSON, *New Jersey Agricultural Experiment Station.*

P24. Excessive stable froth formation in the reticulo-rumen as the primary cause of legume bloat. D. R. JACOBSON, L. D. BROWN, D. R. DOWDEN, F. H. BAKER, AND R. B. GRAINGER, *University of Kentucky.*

P25. Bloat. Results from various drenchings, including effectiveness of penicillin for prevention. C. L. MOORE, V. A. HALL, AND A. E. DRACY, *South Dakota Agricultural Experiment Station.*

P26. Effects of hot weather on milk production and forage consumption of Holstein cows. J. E. JOHNSTON, E. J. STONE, J. W. SMITH, G. SCHRADER, AND J. B. FRYE, JR., *Louisiana State University.*

P27. The effect of concentrate intake at calving time on physiological activities with special emphasis on ketosis. H. D. Hathaway, W. J. BRAKEL, W. J. TYZNIK, AND H. E. KAESER, *The Ohio Agricultural Experiment Station* and *The Ohio State University.*

P28. Energy utilization and heat tolerance in Indian and European cattle. H. H. KIBLER. A. C. RAGSDALE, AND H. D. JOHNSON, *University of Missouri.*

P29. Blood volumes of dairy calves comparing Cr^{51}-tagged red blood cells and T-1824 plasma dilution methods. P. R. STAHL AND H. E. DALE, *University of Missouri.*

P30. The recovery of milk and blood levels of aureomycin when fed to lactating dairy cows. B. W. HENDERSON, JR., AND J. W. COBBLE, *University of Rhode Island.* H. L. EASTERBROOKS, *American Cyanamid Company, Pearl River, New York.*

2:00–4:00 P.M.

JOINT WITH EXTENSION AND MANUFACTURING SECTIONS

Selected Topic: Teaching Methods

(SEE EXTENSION SECTION)

Student Union Basement, Room 2

Friday, June 28

8:00–11:15 A.M.

SECTION A. **Silage and Pasture**

S. W. MEAD, *Chairman*

Classroom Building Auditorium

P31. Comparison of the nutritive evaluations of roughage feeds by observation with chemical determinations. J. W. BRATZLER, E. KECK, JR., AND R. W. SWIFT, *Pennsylvania State University.*

P32. Relative feeding value of corn and sorghum silage. J. R. OWEN, J. T. MILES, W. C. COWSERT, J. W. LUSK, AND E. W. CUSTER, *Mississippi State College.*

P33. Comparison of oat silage and corn silage as roughages for milking cows. C. A. LASSITER, C. F. HUFFMAN, C. W. DUNCAN, AND S. T. DEXTER, *Michigan State University.*

P34. Diurnal excretion of chromium oxide and ash by dairy cows. P. A. PUTNAM, J. K. LOOSLI, R. G. WARNER, AND S. R. SEARLE, *Cornell University.*

P35. Observations on the application of the chromic oxide technique to pasture nutrition studies. F. R. MURDOCK, A. S. HODGSON, V. L. MILLER, AND F. KIMURA, *State College of Washington, Western Washington Experiment Station.*

P36. Methoxyl content of forage and feces as indirect indicators of dry matter digestibility. C. R. RICHARDS AND H. G. WEAVER, *University of Delaware.*

P37. Some characteristics of alfalfa silages made in unpacked stacks covered with polyethylene film. B. J. WALKER, D. L. HILL, C. H. NOLLER, AND N. S. LUNDQUIST, *Purdue University.*

P38. The improvement of forage preservation in bunkers and stacks through the use of temporary seals. C. H. GORDON, *Dairy Husbandry Research Branch*, and J. R. MCCALMONT, *Agricultural Engineering Research Branch, USDA, Beltsville, Maryland.*

P39. Run-off losses in high-moisture grass silage and effects of recirculating run-off. J. D. HANSEN, R. P. NIEDERMEIER, G. P. BARRINGTON, AND C. E. ZEHNER, *University of Wisconsin.*

P40. A comparison of soilage (chopped fresh alfalfa) and alfalfa silage for lactating cows. C. F. FOREMAN, R. S. ALLEN, AND A. R. PORTER, *Iowa State College.*

P41. The nutritive value of oat silages made from plants ensiled in the boot, milk, and hard-dough stages of maturity. O. T. STALLCUP AND O. H. HORTON, *Arkansas Agricultural Experiment Station.*

P42. Proportions of hay and silage in the dairy ration. A. D. PRATT AND H. R. CONRAD, *Ohio Agricultural Experiment Station.*

8:00–11:15 A.M.

SECTION B. **Artificial Insemination**
S. P. MARSHALL, *Chairman*
Classroom Building, Room 112

P43. The repeatability of natural service (NS) and artificially sired (AB) daughters on subsequent AB performance as measured by regressions of daughter averages. A. G. SENDELBACH, E. L. CORLEY, AND E. E. HEIZER, *University of Wisconsin.*

P44. Effects of pH, osmotic pressure, and bulk cations on the metabolic activity of bull sperm. R. G. CRAGLE AND G. W. SALISBURY, *University of Illinois.*

P45. Effects of glycine and glycerol on the metabolic activity of bovine spermatozoa. R. J. FLIPSE AND J. O. ALMQUIST, *Pennsylvania Agricultural Experiment Station.*

P46. Fertility of bull semen extended with glycerinated, heated, homogenized milk. J. A. WILLIAMS, *Michigan State University.* R. W. GREEN AND F. DOMBROSKE, *Michigan Artificial Breeders Cooperative.*

P47. Autoradiography of bovine spermatozoa with glycerol-1-C^{14}. B. W. PICKETT AND C. P. MERILAN, *University of Missouri.*

P48. Preliminary fertility results from the preservation of bovine semen at room temperature. N. L. VANDEMARK AND U. D. SHARMA, *University of Illinois.*

P49. Relation of testicular size to production of spermatozoa by young and aged bulls. E. L. Willett and J. I. Ohms, *American Foundation for Biological Research, Madison, Wisconsin.*

P50. Tenure of dairy bulls in artificial service. R. B. Becker and P. T. Dix Arnold, *Florida Agricultural Experiment Station.*

P51. Rapid rates for freezing bull semen. G. Bialy and V. R. Smith, *University of Wisconsin.*

P52. Semen freezing apparatus using cold, flowing alcohol. M. H. Ehlers and J. Rice, *State College of Washington.*

P53. Variations in quality of liquid and frozen semen at various temperatures. D. F. Weseli, T. M. Ludwick, E. A. Hess, and F. Ely, *The Ohio State University* and *Ohio Agricultural Experiment Station.*

P54. A study of bull stud records to determine the effect of varying frequencies of ejaculation on semen production, quality, and fertility. E. C. Frederick and E. F. Graham, *University of Minnesota.*

P55. The effect of plane of nutrition and frequency of collection on bovine semen production. E. F. Graham and E. C. Frederick, *University of Minnesota.*

P56. Effect of management practices upon sperm production of young dairy bulls. L. J. Boyd and N. L. VanDemark, *University of Illinois.*

9:00–11:00 A.M.

Education Committee (Joint Manufacturing, Production, and Extension Sections)

Selected Topic: Curriculum and Course Organization for Dairy Science

(See Manufacturing Section)

Student Union Pioneer Room

11:30 A.M.–12:30 P.M.

Production Section Business Meeting

S. W. Mead, *Chairman*

Classroom Building Auditorium

1:30–4:00 P.M.

Section A. **Milk Secretion**

S. W. Mead, *Chairman*

Classroom Building Auditorium

P57. Volatile fatty acids (VFA) of milk fat and milk fat secretion. S. Lakshmanan, *University of Maryland.*

P58. The influence of environmental temperature on milk composition. C. P. Merilan and K. W. Bower, *University of Missouri.*

P59. Normal variations in the maximum rate of milk flow in Holstein-Friesian dairy cattle. E. W. Hupp and M. Plum, *University of Nebraska.*

P60. Biological assay of milk for estrogenic activity. C. W. Turner, *Missouri Agricultural Experiment Station.*

P61. Estimation of estrogen secretion in dairy cows in late pregnancy. T. El Attar and C. W. Turner, *Missouri Agricultural Experiment Station.*

P62. Bioassay of milk for estrogenic activity from cows implanted with diethylstilbestrol. H. H. Voelker and E. R. Becker, *South Dakota State College.*

P63. Effects of cortisone on lactation and involution of mammary glands in rats. R. M. Johnson and J. Meites, *Michigan State University.*

P64. Origin of the major specific proteins in milk. B. L. Larson and D. C. Gillespie, *University of Illinois.*

P65. Effect of growth hormone and ACTH on secretion of milk fat. A. C. Chung and M. Keeney, *University of Maryland.*

P66. Effects of feeding thyroprotein for a ninety-day period. R. G. Hinders and G. M. Ward, *Colorado State University.*

1:30–4:00 P.M.

Section B. **Calf Nutrition**
S. P. Marshall, *Chairman*
Classroom Building, Room 112

P67. A comparison of alfalfa hay and wilted alfalfa silage as roughage for growing dairy calves. J. W. Thomas, J. F. Sykes, and L. A. Moore, *Dairy Husbandry Research Branch, USDA, Beltsville, Maryland.*

P68. Effect of age and of dietary regime on hemoglobin and reducing sugar levels in the blood of dairy calves. L. Ratcliff, N. L. Jacobson, and R. S. Allen, *Iowa State College.*

P69. The utilization of intravenously administered carotene by dairy calves. J. D. Schuh, M. Ronning, and W. D. Gallup, *Oklahoma A & M College.*

P70. Effects of fat supplements in a simple milk replacer on scours in dairy calves. R. E. Leighton and R. Reiser, *Texas Agricultural Experiment Station.*

P71. Interrelationships of milk temperature, dilution, and curd formation in the response of calves to whole milk diets. F. G. Owen and C. J. Brown, *Arkansas Agricultural Experiment Station.*

P72. Effects on dairy calves of feeding chlortetracycline and of removing it from the ration. S. P. MARSHALL, J. M. WING, AND P. T. DIX ARNOLD, *Florida Agricultural Experiment Station.*

P73. Nitrogen retention studies with dairy calves. J. L. GRIFFITH, E. W. HUMBLE, AND R. J. WILSON, *Oklahoma A & M College.*

P74. The susceptibility of intestinal bacteria to phagocytosis as affected by antibiotics. D. L. MACFADDEN AND E. E. BARTLEY, *Kansas State College.*

P75. Performance of dairy calves fed high-roughage pellets containing different levels and sources of protein. J. W. HIBBS AND H. R. CONRAD, *Ohio Agricultural Experiment Station.*

4:00–5:00 P.M.

JOINT WITH EXTENSION SECTION

Joint Committee Reports

S. W. MEAD, *Chairman*

Classroom Building Auditorium

Breeds Relation. K. A. HUSTON, *Kansas*

Dairy Cattle Breeding. S. N. GAUNT, *Massachusetts*

Dairy Cattle Type. C. D. MCGREW, *Ohio*

Dairy Cattle Health. C. G. BRADT, *New York*

5:00 P.M.

JOINT WITH EXTENSION AND MANUFACTURING SECTIONS

Selected Topic: Solids-Not-Fat and Its Constituents

S. N. GAUNT, *Chairman*

Classroom Building, Room 112

5:00 P.M.

JOINT WITH EXTENSION SECTION

MEETING OF SUPERINTENDENTS OF OFFICIAL TESTING

(SEE EXTENSION SECTION)

Classroom Building Auditorium

Saturday, June 29

8:00–10:15 A.M.

SECTION A. **Reproduction**

S. W. MEAD, *Chairman*

Classroom Building Auditorium

P76. The duration of estrus and the length of estrous cycles in dairy cattle in a subtropical climate. C. BRANTON, J. G. HALL, E. J. STONE, R. B. LANK, AND J. B. FRYE, JR., *Louisiana Agricultural Experiment Station.*

P77. Effects of preceding superovulation with exogenous progesterone. J. R. NICHOLS, P. J. DZUIK, T. AVERY, AND W. E. PETERSEN, *University of Minnesota.*

P78. Effect of progesterone on the incidence of retained placenta. R. L. HAYS AND L. E. MCDONALD, *University of Illinois.*

P79. The effects of iso-immunization with blood and semen on fertility in cattle. C. A. KIDDY, W. H. STONE, W. J. TYLER, AND L. E. CASIDA, *University of Wisconsin* and *Dairy Husbandry Research Branch, USDA.*

P80. Effect of breed, season, and age on the interval of calving to first heat. R. L. HAYS, *University of Illinois.*

P81. Fertility rates among normal and abnormal cows bred following estrous cycles of varying length. R. E. ERB AND M. H. EHLERS, *State College of Washington.*

P82. Carotene requirements of Holstein cows. MAGNAR RONNING, E. R. BEROUSEK, AND W. D. GALLUP, *Oklahoma A & M College.*

P83. Some relationships between twin births and milk production and breeding efficiency. W. H. CLONINGER AND H. W. THOELE, *The Pennsylvania State University.*

8:00–10:15 A.M.

SECTION B. **Genetics**
S. P. MARSHALL, *Chairman*
Classroom Building, Room 112

P84. Repeatability of teat measurements in dairy cattle at various ages. D. O. RICHARDSON, T. M. LUDWICK, H. E. RICKARD, AND W. M. ETGEN, *The Ohio State University* and *Ohio Agricultural Experiment Station.*

P85. Use of extended incomplete lactation records. H. H. VOELKER, *South Dakota State College.*

P86. Heritability of fat yields in herds with different production levels. J. E. LEGATES, *North Carolina State College.*

P87. Repeatability of dairy cow butterfat records in New Zealand. O. M. CASTLE AND S. R. SEARLE, *New Zealand Dairy Board, Wellington.*

P88. Influence of season of the year and sire upon the birth weight and the six- and twelve-month gains in weights for Holstein females. H. P. DAVIS, *University of Nebraska.*

P89. Some changes and correlations of type ratings in immature Holstein females. C. J. WILCOX, K. O. PFAU, AND J. W. BARTLETT, *Rutgers University, Sussex, New Jersey.*

P90. Heritability and phenotypic and genetic correlations between type ratings and milk and butterfat production in Holstein-Friesian cattle. R. G. MITCHELL, E. L. CORLEY, E. E. HEIZER, AND W. J. TYLER, *University of Wisconsin.*

9:00–10:15 A.M.

STUDENT AFFILIATES AND FACULTY ADVISORS' MEETING
(SEE MANUFACTURING SECTION)
Student Union Corral Room

10:30 A.M.–12:30 P.M.

GENERAL BUSINESS SESSION OF THE ASSOCIATION
C. F. HUFFMAN, *Chairman*
Student Union Ballroom

2:00–5:00 P.M.

JOINT WITH MANUFACTURING AND EXTENSION SECTIONS
Symposium: Legal and Regulatory Aspects of Dairy Products Manufacture
(SEE MANUFACTURING SECTION)
Student Union Ballroom

2:00–5:30 P.M.

SECTION A. **Rumen Physiology**
S. W. MEAD, *Chairman*
Classroom Building Auditorium

P91. Rumen perfusion as a technique for measuring absorption of rumen substances and observations on volatile fatty acid levels in blood. R. D. MCCARTHY, J. C. SHAW, F. G. HUETER, AND J. MCCARTHY, *University of Maryland.*

P92. In vitro studies on methanogenic rumen bacteria. Utilization of valeric and butyric acids. R. A. OPPERMANN, W. O. NELSON, AND R. E. BROWN, *University of Illinois.*

P93. Effect of frequency of feeding on animal response, feed digestibility, and rumen activity. A. H. RAKES, W. A. HARDISON, J. ALBERT, W. E. C. MOORE, AND G. C. GRAF, *Virginia Agricultural Experiment Station.*

P94. Effect of hay-concentrate ratio on rumen fill as measured by an antipyrine dilution technique. R. S. EMERY, C. K. SMITH, AND T. R. LEWIS, *Michigan State University.*

P95. In vitro digestion of carbohydrates by rumen microorganisms. E. CHENG AND W. BURROUGHS, *Iowa Agricultural Experiment Station.*

P96. The effect of chlortetracycline feeding on in vivo cellulose digestion by rumen microorganisms. M. R. LAMBERT AND N. L. JACOBSON, *Iowa State College.*

P97. Effect of aureomycin on volatile fatty acid production by rumen microorganisms. E. C. LEFFEL, W. H. BROWN, AND S. LAKSHMANAN, *University of Maryland.*

P98. Influence of excess dietary protein intake on rumen volatile fatty acids, in vitro cellulose digestion, and blood glucose and ketones in lactating cows. J. F. STEIN AND E. M. KESLER, *Pennsylvania State University.*

P99. The influence of added lignin on the digestion of cellulose in an artificial rumen and on the digestibility of nutrients when added to the ration of dairy cattle. O. T. STALLCUP, *Arkansas Agricultural Experiment Station.*

P100. Growth response of bovine rumen bacteria to single amino acids as the sole nitrogen source. J. J. GILROY AND F. G. HUETER, *University of Maryland.*

P101. Purine dissimilation by bovine rumen WCS. R. N. DOETSCH AND P. JURTSHUK, *University of Maryland.*

P102. Obligate anaerobic urea-hydrolyzing bacteria in the bovine rumen. R. J. GIBBONS AND R. D. MCCARTHY, *University of Maryland.*

P103. Ruminant metabolism on diets producing a low-fat-content milk. J. C. SHAW, E. C. LEFFEL, R. N. DOETSCH, T. R. LEWIS, R. R. ROBINSON, M. E. SENGER, AND W. H. BROWN, *University of Maryland.*

EXTENSION SECTION

All Meetings in Student Union Basement, Rooms 1, 2, 3

Thursday, June 27

8:30–9:30 A.M.

DAIRY RECORDS COMMITTEE MEETING
Student Union Corral Room

2:00–4:00 P.M.

JOINT WITH PRODUCTION AND MANUFACTURING SECTIONS
Selected Topic: Teaching Methods
J. D. BURKE, *Chairman*
Student Union Basement, Room 2

Visuals and You. D. T. SCHILD, *Division of Information Programs, USDA.*

E1. Cow colleges: A new approach to winter meetings. R. W. SPALDING, *Cornell University.*

E2. A feeding school in every county on the same day. J. G. CASH, *University of Illinois*

4:30–5:00 P.M.

Presentation and Discussion of Exhibits
L. H. STINNETT, *Chairman*
Student Union Basement, Rooms 1, 2, 3

Friday, June 28

9:00–11:00 A.M.

EDUCATION COMMITTEE (JOINT MANUFACTURING, PRODUCTION, AND EXTENSION SECTIONS)

Selected Topic: Curriculum and Course Organization for Dairy Science

(SEE MANUFACTURING SECTION)

Student Union Pioneer Room

9:00–11:15 A.M.

Milk Records and Herd Improvement

J. D. BURKE, *Chairman*

Student Union Basement, Room 2

E3. Milk records as a basis of herd improvement. G .W. SALISBURY, *University of Illinois.*

E4. Three years' experience with Weigh-A-Day-A-Month. J. G. CASH, *University of Illinois.*

Weighing and Sampling Devices for Pipeline Milkers. J. H. BOOK, *Dairy Husbandry Research Branch, UDSA.*

Mechanical and Electronic Processing of Herd Improvement Records. J. F. KENDRICK, *Dairy Husbandry Research Branch, USDA.*

11:15 A.M.–12:30 P.M.

EXTENSION SECTION BUSINESS MEETING

J. D. BURKE, *Chairman*

Student Union Basement, Room 2

Report of Dairy Records Committee. J. W. CROWLEY, *Chairman*
Report of 4-H Club Committee. E. R. BONEWITZ, *Chairman*
Report of Resolutions Committee. I. E. PARKIN, *Chairman*
Report of Teaching Methods Committee. L. H. STINNETT, *Chairman*
Report of Nominating Committee.

1:30–4:00 P.M.

JOINT WITH PRODUCTION AND MANUFACTURING SECTIONS

(SEE PRODUCTION AND MANUFACTURING, SECTIONS A AND B)

4:00–5:00 P.M.

JOINT WITH PRODUCTION SECTION

Joint Committee Reports

(SEE PRODUCTION SECTION)

Classroom Building Auditorium

5:00 P.M.

JOINT WITH PRODUCTION AND MANUFACTURING SECTIONS

Selected Topic: Solids-Not-Fat and Its Constituents

(SEE PRODUCTION SECTION)

Classroom Building, Room 112

5:00 P.M.

JOINT WITH PRODUCTION SECTION
MEETING OF SUPERINTENDENTS OF OFFICIAL TESTING
K. A. HUSTON, *Chairman*
Classroom Building Auditorium

Saturday, June 29

9:00–10:15 A.M.

STUDENT AFFILIATES AND FACULTY ADVISORS' MEETING
(SEE MANUFACTURING SECTION)
Student Union Corral Room

9:00–10:30 A.M.

HERD MANAGEMENT PHASE OF THE 4-H DAIRY PROJECT
L. A. JOHNSON, *Chairman*
Student Union Basement, Room 2

E5. Contributions of the Dairy Breed Associations and the Purebred Dairy Cattle Association to 4-H. J. E. COOPER, *Director of Extension, The Holstein-Friesian Association of America.*

E6. The Wisconsin herd management 4-H dairy project. M. E. NELSON, *University of Wisconsin.*

E7. 4-H dairy herd management project in Missouri. F. H. MEINERSHAGEN, *University of Missouri.*

E8. 4-H dairy herd record project. J. D. GEORGE, *North Carolina State College.*

E9. Report of the National 4-H Club Dairy Development Committee. C. C. OLSON, *North Dakota College of Agriculture.*

Installation of Officers.

Committee Announcements.

10:30 A.M.–12:30 P.M.

GENERAL BUSINESS SESSION OF THE ASSOCIATION
C. F. HUFFMAN, *Chairman*
Student Union Ballroom

2:00–5:00 P.M.

JOINT WITH MANUFACTURING AND PRODUCTION SECTIONS
Symposium: Legal and Regulatory Aspects of Dairy Products Manufacture
(SEE MANUFACTURING SECTION)
Student Union Ballroom

2:00–5:00 P.M.

Herd Management and Breeding

L. R. FRYMAN, *Chairman*

Student Union Basement, Room 2

E10. Artificial insemination and trends in dairy herd averages in Virginia. N. R. THOMPSON, V. L. BALDWIN, and G. C. GRAF, *Virginia Polytechnic Institute.*

E11. The effects of some winter management practices and herd size on the 60-90 day nonreturns in artificial breeding. H. J. BEARDEN, *Cornell University.*

E12. Improvement of progeny tests by adjusting for herd, year, and season of freshening. C. R. HENDERSON AND H. W. CARTER, *Cornell University.*

Discussion.

BLOOD LEVELS AND URINARY EXCRETION OF CALCIUM IN NORMAL COWS AND COWS WITH PARTURIENT PARESIS AFTER INTRAVENOUS INJECTIONS WITH CALCIUM BOROGLUCONATE [1, 2, 3]

J. L. ALBRIGHT AND T. H. BLOSSER
Department of Dairy Science
State College of Washington, Pullman

Fourteen cows with milk fever, five normal calving mature cows, and three normal-calving, primiparous heifers were injected with either a 25.5 or 32.7% calcium borogluconate solution. It was found that there was no advantage in treating cows with milk fever with the concentrated solution; that its injection 12 hours post-calving did not prevent milk fever. *Editor.*

Many reports have been published on various types of treatment for cases of parturient paresis (milk fever). Udder inflation *(14, 18)* techniques were the most successful therapy until the classic work of Little and Wright *(15)* demonstrated that a hypocalcemia was associated with milk fever. These investigators *(16)* found that intravenous and intramuscular injections of 30 to 40 ml. of a 10% calcium chloride solution were effective in treating this disorder. One of the disadvantages of using calcium chloride was its toxic effect on tissues. Severe necrosis resulted when solutions of this salt escaped into the muscles. In searching for a form of calcium which could be used advantageously in milk fever, it was found that calcium gluconate $[(C_6H_{11}O_7)_2Ca]$, a neutral organic salt, was well tolerated by the tissues even when injected subcutaneously. Stinson of England *(20)* was the first to use calcium gluconate to treat milk fever. Although this compound became a universal therapy for parturient paresis, it was discouragingly unstable in aqueous solutions, had a low calcium content, and was expensive. Hayden *(13)* and Austin *(1)*, working independently, found that with the addition of boric acid a calcium gluconate solution could be prepared which was stable for months. At present, calcium borogluconate *(3, 4, 9, 11, 17)* is the most widely used material in the treatment of milk fever.

Received for publication October 22, 1956.

[1] Scientific Paper No. 1531, Washington Agricultural Experiment Stations, Pullman. Project 920.

[2] Data contained in this publication are from a thesis submitted by the senior author to the Graduate School of the State College of Washington, in partial fulfillment of the requirements for the degree of Master of Science.

[3] This investigation was supported in part by funds provided by Jensen-Salsbery Laboratories, Kansas City, Kansas.

Recently, a 32.7% calcium borogluconate solution has been prepared by Austin *(2)*. This solution was used on 12 normal cows and found to be more effective in maintaining higher blood serum calcium values over a 24-hour period than a 25.5% calcium borogluconate solution.

This study was initiated to obtain more information on treatment, physiological response to injection, blood serum calcium levels, and urinary calcium excretion following injections of calcium borogluconate (32.7% and 25.5%) in cows afflicted with parturient paresis, normal parturient cows, and primiparous cows.

EXPERIMENTAL PROCEDURE

Three groups of cows from the dairy herd at the State College of Washington were used in this study: one group of 14 cows afflicted with parturient paresis, a second group of five normal parturient cows, and a third group of three primiparous cows. Cows were randomly assigned to injection of either the 32.7 or 25.5% solution.

In the milk fever group, eight cows were treated with the 32.7% solution (containing 2.84% calcium) and six with the 25.5% material (containing 2.22% calcium). When the animal became recumbent due to parturient paresis symptoms, a blood sample was drawn immediately pre-treatment. First treatments for milk fever occurred, on the average, at nine hours post-partum. In treating milk-fever cows, one of the two solutions was injected slowly (approximately in 30 minutes) by intravenous administration at the rate of 0.5 ml. per pound of body weight. At 15 minutes after the conclusion of the injection, a blood sample was taken. It was felt that by 15 minutes post-injection the injected calcium would be well dispersed throughout the animals' circulatory system. Also, by this time, most of the cows treated had arisen.

Blood samples were withdrawn from the external jugular vein every two hours post-injection, for a 24-hour period, then at 36, 48, and 72 hours post-injection. The blood samples were then placed under refrigeration and were subsequently analyzed for blood serum calcium by the Clark and Collip method *(8)*.

The procedure for the normal parturient cows followed the same pattern except for the following points: If the parturient cow was apparently normal up to 12 hours post-calving, she was injected intravenously with one of the two calcium solutions. The logic followed here was that by 12 hours post-partum a potential milk-fever cow would already either have parturient paresis or would have some symptoms of this disorder. That this was an appropriate injection time is indicated by the average post-calving treatment time of nine hours in the milk-fever cows in this study. It was felt that injections at 12 hours post-calving would give comparable calcium metabolism results in normal parturient cows and milk-fever cows.

In the third group, one primiparous animal was injected with the 32.7% material at 12 hours and two at six hours post-calving. Because a slight hypocalcemia existed in the one animal injected 12 hours post-partum, it was thought

possible that the injection of calcium sooner after calving might have a more pronounced hypocalcemic effect. The same blood-sampling intervals that were used in the milk-fever and normal parturient groups were employed.

Urine collections were by the method of Turner *et al. (21)*. In some of the cows, micturition occurred following stimulation, but before treatment, while the cow was still recumbent. In all cases micturition occurred soon after treatment when the cow had arisen in response to treatment. In the normal parturient group, the same practice of stimulating the cow to micturate before injection and immediately post-treatment was used. Urine samples, in both groups, were taken every two hours for a 24-hour post-injection period. Unfortunately, the primiparous animals would not micturate following stimulation. Toluene was added to the urine samples, which were placed under refrigeration. The samples were analyzed for urinary calcium by the method of Morris *et al. (19)* after wet ashing, by a modification of the procedure described by Gerritz *(12)*.

When cows treated with one of the solutions in this study relapsed, the animals were, in most cases, again treated and blood and urine samples collected. Data were not collected following a second relapse into the milk-fever syndrome.

RESULTS AND DISCUSSION

Blood serum and urinary calcium in milk-fever cows. Serum calcium levels for the milk-fever treated group are shown graphically (Figures 1 and 3). As one might expect, the more highly concentrated calcium solution increased the serum calcium considerably above levels of calcium found following injection with the 25.5% solution. Data in Figure 1 shows the serum calcium values at 15 minutes post-treatment to be 25.7 mg. / 100 ml. for the 32.7% material and 21.0 mg. / 100 ml. for the 25.5% solution. By four hours post-treatment the serum calcium levels were approximately 13.6 and 13.7 mg. / 100 ml. for the 32.7 and 25.5% solutions. From four to 24 hours post-treatment the serum calcium levels of the two groups were nearly the same, with less than 1 mg. / 100 ml. difference. It is interesting to note that the serum calcium values were considerably below the normal range of 9 to 12 mg. % toward the end of the 24-hour post-treatment period in both groups. These lower-than-normal calcium values were partially due to the fact that there were several relapses occuring in both groups. Four out of eight cows relapsed following treatment with the 32.7% solution, and three out of six treated with the 25.5% material. An interesting indication was found in the data concerning initial milk-fever attacks and relapses in the same animals. The average serum calcium levels in three cows at the time of milk-fever attack were 5.3 mg. Ca / 100 ml. serum, and 4.7 mg. Ca / 100 ml., at the time of relapse, in the same cows. These data and observations suggest that relapses are more severe than the initial attacks.

However, close examination of the data from individual cows shows that several cows which did not relapse also exhibited lower-than-normal calcium values from 16 to 24 hours post-treatment. If one were to average only these animals which did not relapse, the average calcium values at 24 hours post-treatment

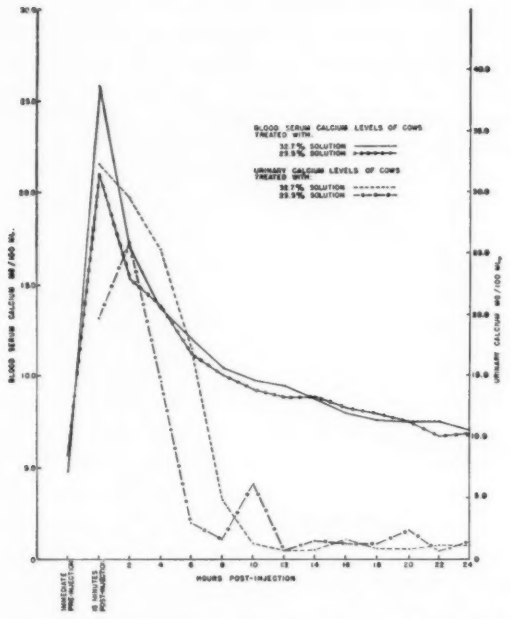

FIG. 1. Blood serum and urinary calcium levels of cows afflicted with parturient paresis subsequent to injection with calcium borogluconate (C.B.G.).

would be 7.8 mg. / 100 ml. for the 32.7% material and 7.5 mg. / 100 ml. for the 25.5% solution. There are two ways of explaining these lower-than-normal blood serum calcium values. The first possibility is that the regulatory system whose failure originally caused the cow to develop milk fever had not completely recovered by 24 hours post-calving. A second possibility is that intravenously injected calcium depresses calcium metabolism. This depression could be due to a disfunction of the parathyroids. Using this hypothesis, one might assume that the factors which tend to increase blood serum calcium levels temporarily inhibit the parathyroids; whereas, those which tend to lower blood serum calcium stimulate the parathyroids. This latter viewpoint has gained significance by the recent work of Boda and Cole *(6, 7)*, in which they fed low-calcium, high-phosphorus diets as a successful preventive for milk fever.

Urinary calcium excretion data for the milk-fever afflicted animals are shown graphically (Figure 1). As might be expected, injection of the 32.7% solution caused the cows to excrete more calcium via urinary channels. In the data available for the 15-minute post-treatment urine samples, the 32.7%-solution treated group excreted 32.4 mg. Ca / ml. urine, while the 25.5% group excreted 19.7 mg. Ca / 100 ml. urine. At two hours post-treatment, the calcium levels were 29.5 mg. / 100 ml. urine and 25.9 mg. / 100 ml. urine for the 32.7 and 25.5% solutions, respectively. One puzzling feature was the large average amount

of calcium excreted between eight and and ten hours post-injection in the 25.5% group. This amount resulted from an extremely high value from one cow, which relapsed into the parturient paresis syndrome 14 hours later. If this cow were left out of the group, the average 25.5% group figure would be 1.0 mg. / 100 ml. urine as compared to 1.6 mg. / 100 ml. urine for the 32.7% material. It is interesting to note that by 12 hours post-treatment the calcium concentration of the urine was approximately the same for both groups.

Total urinary calcium excreted following treatment with the two solutions was calculated (calcium concentration × volume). Calcium excretion in animals injected with the 32.7% solution was generally higher throughout the 24-hour collection period than in cows injected with the 25.5% solution. Total calcium excreted in the urine during the 24-hour period for the more concentrated solution was 1,422.5 mg., while the 25.5% material resulted in 1,215.3 mg. excreted per 24-hour period.

Calculations were made based on the percentages of the total dose of calcium excreted via urinary channels following treatment with two solutions. It is realized that this type of calculation represents an oversimplification of existing conditions, for a certain small amount of endogenous calcium is excreted via urinary channels even without calcium injection. Ward *(23)* and Ward *et al. (24)* found that normal parturient cows excreted 510 mg. (range 60 to 980 mg.) of calcium one day post-partum via urinary channels. These investigators also demonstrated that milking cows excreted 75 to 80% and dry cows 99% of the total calcium excreted via the fecal channel.

Calculations based on data available from the study reported herein show that by two hours post-injection the 25.5% group had excreted the equivalent of 7.8% of the total injected calcium, and the 32.7% group had excreted only 4.6%. However, the 32.7% group percentagewise was higher at four, six, and eight hours post-treatment. In the 24-hour period, the group injected with the less concentrated calcium solution excreted 10.8% of the total dose in the urine; whereas, the group injected with the 32.7% solution excreted only 8.5%.

Perhaps the higher values were obtained from the 25.5% solution, because this material was used on animals whose serum calcium prior to injection was substantially higher than that of the 32.7% group. These calcium values were 5.6 mg. / 100 ml. for the cows subsequently injected with the 25.5% solution, and 4.7 mg. / 100 ml. for those injected with 32.7% material. Thus, the animals with the lower serum calcium levels before injection would need more calcium and would be less likely to excrete higher quantities of calcium early in the post-treatment period.

Studies with radio-calcium measure behavior of injected calcium much more exactly. Visek *et al. (22)* studied calcium metabolism by injecting radioactive calcium into normal, lactating, nonparturient goats. He used an average of 1.17 grams per 100 pounds liveweight. This amount was slightly higher than the injections of the 25.5% solution (1.11 gm. / 100 lb. liveweight) and slightly lower than the 32.7% material (1.42 gm. / 100 lb. liveweight) injected into milk-

fever animals in the experiment reported herein. Visek *et al. (22)* recovered 4.66% of the intravenously injected calcium in the urine during a 24-hour post-injection sampling period. As one might expect, this figure is slightly lower than the percentage of injected calcium recovered in the milk-fever cows in this experiment.

Blood serum and urinary calcium in normal parturient cows. Data from the normal parturient cows injected 12 hours post-partum with the two solutions are shown graphically (Figures 2 and 3). The injection of the 32.7 and 25.5% solutions caused an immediate rise in serum calcium. At 15 minutes post-injection, the calcium values were 27.9 mg. and 21.9 mg. / 100 ml. serum for the two compounds, respectively. The levels were higher in the 32.7% group until ten hours post-injection. The calcium levels were in the normal range (9 to 12 mg. %) from eight through 14 hours post-injection in both groups. However, after 14 hours post-injection there was a progressive reduction in serum calcium in the 32.7% group. By 24 hours post-injection these values were considerably below their pre-injection levels.

Two of three normal parturient cows developed milk-fever symptoms 35 hours post-injection or 47 hours post-partum after injections with the 32.7% solution. Ordinarily, one does not expect to find first symptoms of purturient paresis this late after calving. One of the cows had a history of milk-fever

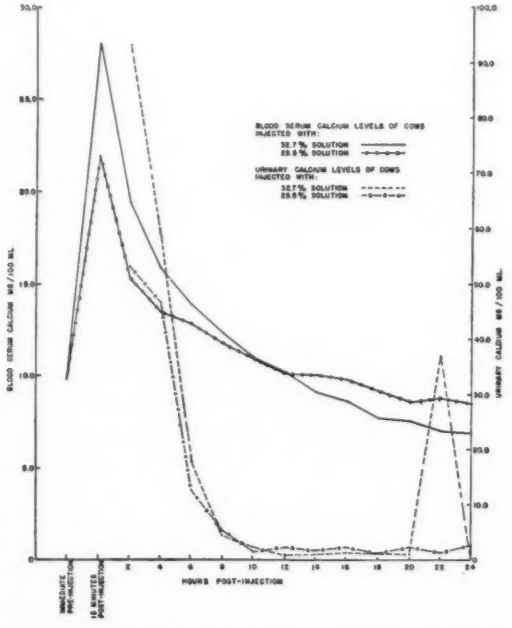

FIG. 2. Blood serum and urinary calcium levels of normal parturient cows subsequent to injection with calcium borogluconate (C.B.G.).

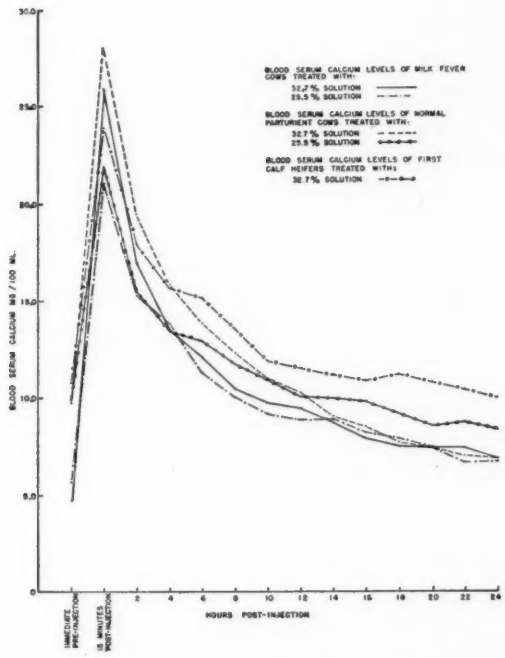

FIG. 3. Blood serum calcium levels of cows afflicted with parturient paresis, normal parturient cows, and primiparous cows, subsequent to injection with calcium borogluconate (C.B.G.).

attack in a previous parturition. The other animal that developed milk fever had never been afflicted with this disorder until the parturition reported herein, Both of the cows developing parturient paresis were in their fourth parturition, while the third normal parturient cow was in her third.

From these limited observations one might infer that large amounts of calcium injected into normal parturient cows depress the parathyroid glands and subsequently cause relapses into the parturient paresis syndrome. However, the number of animals involved in this study is so small that no definite conclusions can be reached.

The 25.5% group maintained relatively normal values throughout the 72-hour post-injection period (Figures 2 and 3). These values were slightly below the pre-injection samples in the period from 16 through 48 hours post-injection. The limited data presented are in slight contrast with Craige *(11)* who injected 500 ml. of a 15% calcium gluconate solution into nonparturient cows. Craige's data show that at 24 hours post-injection the injected cows had essentially the same calcium serum values as they did immediately pre-injection. From this comparison one might suspect that, (1) more highly concentrated calcium solutions have a hypocalcemic effect and / or, (2) that in-

jected calcium elicits a different response in parturient as compared with non-parturient cows.

Data in Figure 2 show that the urinary calcium values were extremely high in both groups following injection with calcium borogluconate. In both groups of normal parturient cows there was a marked diuresis. Recorded calcium values for the 32.7 and 25.5% solutions at two hours post-injection were 93.2 mg. / 100 ml. and 53.5 mg. / 100 ml. urine, respectively. These are much higher levels than were found in milk-fever cows at a comparable time. By eight hours post-injection the urine calcium values of the two groups had declined to approximately the same level. From 20 to 22 hours post-injection the 32.7% group excreted, on the average, extremely high levels of calcium. This average is biased by the results of one cow, which had a calcium excretion value of 110 mg. / 100 ml., compared with 0.4 and 0.6 mg. / 100 ml. in the other two cows. This animal developed parturient paresis symptoms 13 hours after this sample was taken. Blosser and Smith *(5)* state that one cow which subsequently developed milk fever excreted over 3,000 mg. calcium (more than five times normal) via urinary channels during a 24-hour period on the tenth day prepartum.

The average total milligrams of calcium excreted for the 24-hour post-injection period was 3,278.9 mg. for the 32.7% solution and 1,362.8 mg. for the 25.5% material.

Empirical calculations (as previously explained) were made of the percentage of total injected calcium recovered in the urine following injection with the two solutions. In the 32.7% group, 22.4% was recovered by 24 hours post-injection; whereas, in the 25.5% group 12.5% was recovered. These figures are considerably above the figures listed by Visek *(22)* in his studies on the intravenous injection of radio-calcium in goats. These percentage figures are also greater than the previously mentioned calculations that were found in milk-fever treated animals.

Blood serum calcium in primiparous cows. Since parturient paresis rarely occurs in primiparous cows, a probing type of experiment was conducted to obtain additional information concerning the etiology of this disorder. Since normal parturient cows exhibited milk-fever symptoms after injections with 32.7% calcium borogluconate, as reported herein, three primiparous animals were injected with the same solution to check their metabolic response to high levels of calcium.

At 24 hours post-injection one animal showed a slight depression in serum calcium, with a value of 7.5 mg. %. At this time the cow appeared extremely tired, somewhat incoordinated, and exhibited a slight hypocalcemia. The other two animals reacted normally following injection, and showed no signs of physiological stress.

Data of Figure 3 show that starting six hours post-injection, calcium levels are higher for corresponding sampling periods in primiparous cows than in normal parturient or milk-fever animals injected with either the 25.5 or 32.7%

calcium borogluconate. Unfortunately, urine samples were not obtained from the animals in this group.

SUMMARY

Fourteen cows with milk fever, five normally calving mature cows, and three normally calving primiparous heifers were injected with either a 32.7% or 25.5% calcium borogluconate solution. Although cows injected with the more highly concentrated solution responded initially with higher blood serum calcium levels, by four to ten hours post-injection there was little difference in blood serum calcium levels between cows receiving the 32.7% and those receiving the 25.5% solution. Fifty per cent relapses occurred in the milk-fever cows following treatment with each solution. In addition, two of three normal parturient cows injected with the 32.7% solution developed milk fever 35 hours post-injection. From these data it appears that, (a) there is no advantage in treating milk-fever cows with a highly concentrated calcium borogluconate solution and, (b) injection of a highly concentrated calcium borogluconate solution 12 hours post-calving does not prevent milk fever and may actually precipitate attacks.

The data show little, if any, relationships between urinary excretion of calcium following injection of calcium borogluconate and subsequent lowered levels of blood serum calcium and relapses into the milk-fever syndrome.

The three groups of cows (milk-fever, normal parturient, and primiparous) responded in essentially the same way to injection of calcium solutions. Primiparous animals exhibited higher blood serum calcium levels at the end of a 24-hour post-injection period than cows in the other groups.

ACKNOWLEDGMENT

The authors gratefully acknowledge the help of Drs. O. L. Frost, G. H. Keown, R. E. Watts, and D. B. Anderson of the State College of Washington Veterinary Clinic, who assisted in this study.

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VALUE OF CORN AND MILO DISTILLERS FEEDS FOR MILK PRODUCTION

J. K. LOOSLI AND R. G. WARNER
Department of Animal Husbandry, Cornell University, Ithaca, N. Y.

Corn distillers dried grains with solubles, corn distillers dried solubles, milo distillers dried grains with solubles, and milo distillers dried solubles were compared for milk production. Distillers dried grains resulted in more four per cent FCM, as well as milk of a higher fat percentage. The milo distillers feeds were about equal to the corn distillers feeds. *Editor.*

Many dairymen prefer to use corn distillers dried grains with solubles (dark grains) rather than corn distillers grains (light grains), in feeds for milking cows. The value of distillers solubles for poultry and swine, especially as a source of unidentified factors needed in the feed of these nonruminants, suggests the desirability of decreasing the use of solubles for dairy cows. Previous reports *(6, 7)* have indicated that the solubles may be slightly less valuable for milking cows than the distillers grains alone, but the differences were not always statistically significant. An additional experiment has been conducted to obtain more information regarding the value of corn distillers dried solubles and corn distillers dried grains with solubles. At the same time, comparisons were made with milo distillers dried solubles and milo distillers dried grains with solubles, since information regarding the value of these feeds was not found in the scientific literature.

EXPERIMENTAL PROCEDURE

Purebred Holstein-Friesian cows which had recently passed their maximum daily milk yields but had not been rebred were selected for the study. They were fed mixed hay, corn silage, and a concentrate mixture containing 18% of protein during a preliminary period of four to six weeks, to obtain individual records on feed intakes and milk yields. Based on preliminary milk production, the cows were divided into four replicates of four cows each and assigned at random, within replicates, to the dietary treatments specified in a 4×4 Latin-square design. A total of 16 cows was used in the experiment. A mixed hay was offered in sufficient quantities to allow a daily weigh-back of from one to four pounds. The cows were fed all of the corn silage they would clean up. The concentrate mixtures were fed initially at the approximate rate of one pound for each 3.5 lb. of FCM *(1)*. The amounts were adjusted weekly for the decline in milk production, according to the procedure of Lucas *(8)*.

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TABLE 1

Composition of the concentrate mixtures

Ingredients	Mixture No.			
	I	II	III	IV
	(lb.)			
Yellow corn, ground	233	233	233	233
Oats, ground	200	200	200	200
Wheat bran	100	100	100	100
Molasses	50	50	50	50
Corn distillers dried grains with solubles[a]	400	.....	.....	.....
Corn distillers dried solubles[a]	.....	400	.....	.....
Milo distillers dried grains with solubles[b]	.....	.....	400	.....
Milo distillers dried solubles[b]	.....	.....	.....	400
Dicalcium phosphate	10	10	10	10
Salt	7	7	7	7
Total[c]	1000	1000	1000	1000

[a] From a mash bill of 79.0% corn, 11.2% rye, and 9.8% barley malt.

[b] From a mash bill of 87.5% milo, 3% rye, and 9.5% barley malt.

[c] Plus 1 g. of cobalt sulfate per mixture.

The concentrate mixtures were formulated to contain approximately 17% of total protein, using the ingredients shown in Table 1. The federal grade and description of the hay, as well as the chemical composition of individual feeds and concentrate mixtures, are tabulated (Table 2). Chemical procedures used were those described by the AOAC *(2)*. Ether extract of the distillers feeds was determined by the technique of Gustoff and Rasmussen *(5)*. The estimated total digestible nutrient contents of the feeds were calculated using the method of Schneider *et al. (9)*.

Individual records were kept of the daily milk produced and feed consumed. Fat percentage of the milk from each cow was determined each week on composite samples consisting of aliquots from four successive milkings. The cows were weighed on three days at the end of the preliminary and each five-week experimental period. The first experimental period was considered as transitional, and the data from the last four weeks were summarized for comparison.

All data were analyzed statistically, using an analysis of variance technique *(10)*. The multiple range test of Duncan *(3)* was employed in evaluating treatment differences.

RESULTS AND DISCUSSION

Milk production, fat percentage, body weight, and feed efficiency data are shown (Table 3). The average fat percentage of the milk produced on the two rations containing the distillers dried grains was significantly higher ($P < 0.05$) than on the rations containing only the distillers dried solubles. As an average, the two rations containing distillers dried grains resulted in the production of more 4% FCM than those with distillers dried solubles. These differences were statistically significant, as reported in Table 3.

TABLE 2
Chemical composition and estimated TDN content of the feeds

	Moisture	Protein	Ether extract	Fiber	NFE	Ash	TDN
				%			
Mixed hay[a]	7.2	15.5	3.1	20.6	46.4	7.2	58.3
Corn silage	77.0	1.8	0.5	7.0	12.9	0.8	13.3
Corn distillers dried grains with solubles	7.5	27.5	8.7	11.8	39.4	5.1	74.8
Corn distillers dried solubles	7.9	26.0	9.1	2.8	42.5	11.7	77.9
Milo distillers dried grains with solubles	6.5	30.2	8.4	10.5	39.0	5.4	76.3
Milo distillers dried solubles	8.9	22.6	7.5	2.7	43.2	15.1	71.8
Concentrate mixtures containing:							
Corn distillers dried grains with solubles	6.9	16.6	5.3	7.7	58.0	5.5	77.4
Corn distillers dried solubles	7.0	16.9	4.8	4.8	56.9	9.6	75.4
Milo distillers dried grains with solubles	6.7	19.5	5.3	8.0	54.2	6.3	76.0
Milo distillers dried solubles	7.4	16.3	4.6	4.2	57.3	10.2	75.0

[a] Graded as U. S. Sample Grade extra-green mixed hay (moldy) with 76% green color and 28.9% foreign material. The hay consisted of 69.3% clover, 16.8% timothy, and 13.9% alfalfa. The leafness of the clover was 53.6%.

Results on fat test are in agreement with the early work of Armsby and Risser *(1)*, who indicated that distillers dried grains tended to increase the fat percentage of milk. These results also confirm the small average increase in FCM and fat percentage previously noted *(4, 5)* for mixtures containing corn distillers dried grains over those containing corn distillers dried solubles. Heretofore these increases have not been consistent enough to be statistically significant.

The small differences in body weight, average daily gain, and feed efficiency between rations were not significant. The average daily feed intakes are reported

TABLE 3
Average daily milk and fat production, body weights, and feed efficiency

Mixture containing	Average daily				Body weight	TDN per lb. FCM
	Milk	Fat	FCM	Gain		
	(lb.)	*(%)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*
Corn distillers dried grains with solubles	44.4	3.63[a]	41.9[b]	0.57	1295	0.61
Corn distillers dried solubles	43.3	3.47	39.9	0.64	1282	0.62
Milo distillers dried grains with solubles	44.0	3.62[a]	41.5[c]	0.80	1290	0.60
Milo distillers dried solubles	42.4	3.52	39.1	0.33	1299	0.62

[a] Significantly greater than the two other mixtures.
[b] Significantly greater than the two distillers dried solubles rations ($P < 0.05$).
[c] Significantly greater than the milo distillers dried solubles ration ($P < 0.05$).

TABLE 4
Average daily feed intakes

Mixture containing	Average daily intake		
	Concentrate	Hay	Silage
	(lb.)		
Corn distillers dried grains with solubles	14.1	12.2	45.6
Corn distillers dried solubles	13.9	12.4	45.0
Milo distillers dried grains with solubles	14.3	12.1	47.6
Milo distillers dried solubles	14.2	12.0	44.7

(Table 4), and because of the method of feeding were similar. There was no evidence to indicate that any of the mixtures were unpalatable.

It is evident from this and earlier studies *(6, 7)* that the unidentified factors which distillers dried solubles furnish to poultry and swine have little or no special value for dairy cows consuming liberal amounts of hay and silage. In fact, it appears that the distillers dried solubles per se may reduce the value of the distillers dried grains for milk production. Earlier studies *(6)* have shown, however, that under the conditions of our experiments, distillers dried solubles are fully equal to rye distillers dried grains with solubles, or a mixture of corn gluten feed and solvent extracted soybean oil meal.

It is noteworthy that the data for milk production and fat test on the milo distillers feeds were similar to those on the corn distillers feeds, suggesting that they have approximately the same value.

SUMMARY

An experiment involving 16 lactating dairy cows was conducted to compare the value for milk production of corn distillers dried grains with solubles, corn distillers dried solubles, milo distillers dried grains with solubles, and milo distillers dried solubles. Feeds containing the distillers dried grains resulted in the production of significantly more four per cent FCM, as well as of milk of a higher fat percentage. The milo distillers feeds were approximately equal to the corn distillers feeds.

ACKNOWLEDGMENTS

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TECHNIQUE FOR MEASURING ERUCTATED GAS IN CATTLE[1]

H. W. COLVIN, JR.,[2] J. D. WHEAT,[3] E. A. RHODE,[3] AND J. M. BODA
Department of Animal Husbandry, University of California, Davis

To measure eructated gas, a mask was put on the animal's muzzle and a cannula inserted at the anterior end of the transected trachea. A tube connected the mask and cannula to a spirometer. The gas escaped via the trachea. The values obtained with the eructation technique agreed closely with those obtained by aspirating the gas directly from the rumen. *Editor.*

Bloat in ruminants has been associated with the cessation, or at least hindrance, of eructation *(4)*. Although it was recognized by Wester *(23)* and Schalk and Amadon *(20)* that rumen motility and eructation were closely related, only a limited amount of work has been done on the physiological and anatomical events taking place during eructation *(4)*.

Dougherty and Meredith *(12)*, in their recent cinefluorographic studies, have illustrated eructation in a normal sheep. In a later paper, the events were described in detail *(11)*.

It has been shown by Dziuk and Sellers *(11)* that the vagus nerves contain branches associated with eructation. These workers caused an increase in eructation rate in cattle by stimulating cutaneous leads of silver electrodes implanted in the thoracic vagus trunks. Cole *et al. (6)* suggested that scabrous material in the diet produced the effective stimulus for eructation. Weiss *(22)* maintains that in sheep, pressure in the posterior dorsal sac supplies the necessary stimulus. In support of the latter premise, Iggo *(15)* has noted an increase in the frequency of impulses traversing a branch of the vagus in sheep and goats as a result of artificial stretch applied to the rumen wall. All of the foregoing studies, plus the many bloat experiments conducted, have been hampered by lack of a method for determining when an animal eructates and the volume of gas per eructation.

It is the purpose of this paper to describe a successful technique for the measurement of eructated gas in cattle.

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[1] This paper was prepared from data presented in a thesis by the senior author in partial fulfillment of requirements for the degree of Doctor of Philosophy at the University of California, Davis, California.

[2] Present address: Department of Physiology and Pharmacology, Oklahoma A and M College, Stillwater, Oklahoma.

[3] School of Veterinary Medicine, University of California, Davis, California.

EXPERIMENTAL PROCEDURES

The original plan of establishing an esophageal fistula, into which would be placed a cannula for gas withdrawal, was abandoned because of the potential interference with deglutition and regurgitation. Instead, a fistula was made in a steer, in the ventral aspect of the trachea slightly posterior to the larynx. A curved plastic cannula, whose outside diameter corresponded to the inside diameter of the trachea, was inserted so that the respired gas was prevented from going into the mouth and nose. Over the muzzle of the steer was placed a modified Edick mask *(16)*, which was connected to a spirometer. (It was expected that the eructated gas would come up the esophagus and pass out into the mask and then on to the spirometer.)

During preliminary trials, it was observed that at the time of each eructation, rumen gas escaped from around the tracheal cannula. Eructated gas was coming up the esophagus and was passing through an open glottis down into the trachea. Attempts were made to prevent the escape of the eructated gas by inflating a toy balloon in the trachea, anterior to the fistula site. In order to prevent stimulating the cough reflex, the balloon was inflated only enough to occlude the trachea. During the experiments which followed, the balloon was blown posteriorly and out of the fistula with each eructation. This permitted contamination of the eructated gas with respired gas. Furthermore, most of the eructated gas escaped via the tracheal fistula.

Daniel *(9)* and Clagett *et al. (3)*, as quoted by Markowitz *(17)*, indicated that tracheal resection could be carried out on dogs without undue stress. On the basis of these reports, tracheal transections were carried out on seven steers and two cows whose average body weight was 1,000 lb. By suturing the ends of the trachea individually to the skin, the separation of rumen and respired gas was assured.

Steps involved in the treatment of each animal before, during, and after tracheal surgery were as follows: *(a)* Food and water were withheld for 24 hours. *(b)* The animal was fixed on an hydraulic large-animal table in the left lateral recumbency and the surgical field was clipped, shaved, and swabbed with tincture of Zephiran (Winthrop-Stearns). *(c)* A sedative dose of chloral hydrate was administered. *(d)* One-half grain of atropine was injected subcutaneously, to prevent excessive salivation. *(e)* The proposed incision line was infiltrated with 2.0% procaine hydrochloride solution. *(f) Surgery: (1)* Using a lateral approach, an incision ten inches long was made six inches posterior to the larynx, over the separation between the sternomandibularis and sternomastoideus muscles. *(2)* By blunt dissection the muscles were separated until the trachea was exposed. *(3)* The trachea was separated and lifted up from the surrounding tissues. *(4)* The exteriorized trachea was transected midway between the exposure. *(5)* At both ends of the incision, circular sections of skin, which conformed to the outside diameter of the transected trachea, were removed. *(6)* The severed ends of the trachea were placed in the respective areas cut for them in the skin, and sutured in place with No. 3 Dermalon (Davis

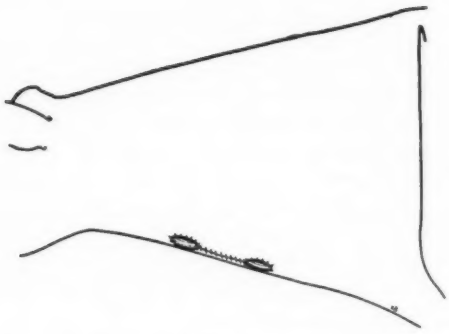

FIG. 1. Site of the completed tracheal transection operation. A curved cannula was inserted in the caudal portion of the transected trachea to prevent suffocation resulting from the constriction of the skin around the opening.

& Geck). (7) The remainder of the incision line was closed. (8) Terramycin (Pfizer) suspension was infused in the incision area. (9) The position of the completed operation is indicated (Figure 1).

(g) Post-operative care: (1) Three million units of procaine penicillin G (aqueous solution) were injected intra-muscularly daily for three days. *(2)* Two weeks following the operation the sutures were removed.

In the posterior end of the trachea, an acrylic plastic cannula with a 155-degree angle was inserted. The outside diameter of the cannula was approximately equal to the inside diameter of the trachea. It was necessary to change the cannulae once a week or more often, since they became clogged with layers of dried mucus and blood.

Since the caudal tracheal cannulae could be coughed out with ease, it was necessary to hold them in place by using a strap (2½-inch cotton and nylon webbing) around each animal just posterior to the shoulders. On each side, a hole was drilled in the exposed part of the cannula. Heavy rubber bands were placed in these holes and stretched back toward the shoulder strap. A piece of heavy copper wire covered with plastic joined each rubber band to the shoulder strap. Such an arrangement permitted freedom of movement for the animals, yet held the cannulae in place.

Having their tracheas transected did not appear to affect the behavior or habits of the animals. All remained on feed, ruminated, and eructated regularly. Respiration appeared to be unimpaired. However, only five of the animals operated on survived long enough to be used in experiments. These deaths can not be attributed to the surgical technique, because the suture lines, trachea, and lungs appeared free of any complications at necropsy; in every case the cause of death was suffocation resulting from accidental dislodging of a caudal tracheal cannula.

When a caudal tracheal cannula was dislodged, immediately the surrounding area began to constrict. At the same time, there was a tendency for the skin around the posterior end of the trachea to fold over the opening. During inspiration these folds would be drawn down against the orifice like a cap. If this condition was not corrected within a short time, death followed rapidly. It is believed that the use of flexible plastic tubing, which would connect the two ends of the transected trachea, should alleviate these losses.

Permanent rumen cannulae were installed according to Quin *et al.* (*19*). These workers utilized cotton wool to hold each cannula in place; in the present study a rubber washer was slipped down over the external portion of the cannula and a plastic flange cemented above this. When used during an experiment, the rubber washer was slipped down the cannula next to the skin, in order to form an air-tight seal. The cannulae were constructed of acrylic plastic with an inside diameter of one-half inch. This type of cannula has recently been described by Dougherty *(10)*.

Figure 2 illustrates schematically the arrangement of the apparatus for collecting and measuring belched gas. The Edick mask *(16)*, which was placed over the experimental animal's muzzle, was modified by removing the air-intake valves and soldering sheet-metal plates in their place, to make the mask air-tight.

A plastic cannula with an inflatable rubber cuff (Figure 3) was inserted in the cephalic end of the transected trachea. When inflating the cuff, it was found that too much pressure impeded eructation: therefore, precaution was taken to inflate the cuff only enough to form an air-tight barrier between the tracheal wall and the cannula.

The interference with eructation probably involved a mechanical factor. Because the tracheal wall is a rigid structure, when the cuff of the cannula was inflated it spread both posteriorly and anteriorly, filling the space between the cannula and the tracheal wall. Over-inflation probably caused the cuff to

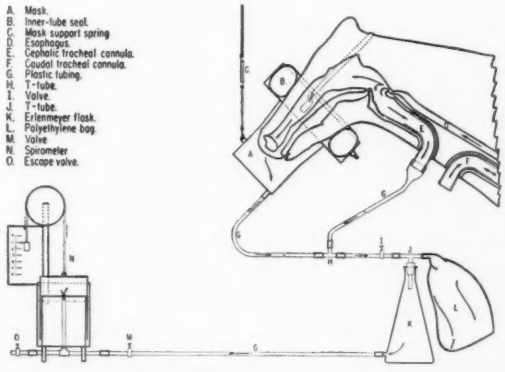

FIG. 2. A schematic diagram of the apparatus used to collect and measure eructated gas. In this type of preparation, the animal respires through the caudal tracheal cannula (F).

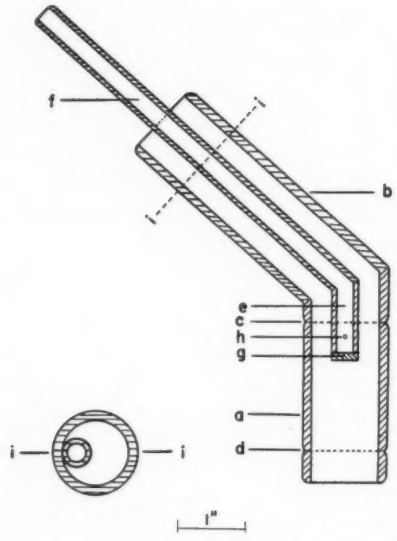

FIG. 3. A scale diagram of a cephalic tracheal cannula. The inflatable cuff is not shown. These cannulae were constructed of acrylic plastic tubing of various sizes. The following is a key to the letters indicated in the diagram: (a) Portion of the cannula which is inserted into the cephalic transected trachea: (b) exterior part of the cannula: (c) and (d) grooves in the cannula for the rubber bands which fit over the rubber cuff and make it air-tight; (e) and (f) parts of the valve stem; (g) solid piece of plastic cemented to end of stem to prevent escape of air; (h) hole joining interior of valve and area immediately beneath the rubber cuff fitted over the outside of the cannula; (i) cross-section of the cannula. The solvent and cement for acrylic plastic is ethylene dichloride. After inflation of the cuff, a short piece of rubber tubing was stretched over the valve stem (f), folded back on itself, and held fast with a small rubber band.

spread over the anterior end of the cannula, causing a mechanical blockage of the gas passageway.

Referring to Figure 2, the plastic tubings (G) (5/8-inch, inside diameter) leading from the mask and the cephalic tracheal cannula were connected to separate ends of a T-tube (H). The other end of the T-tube was attached to a valve (I), which consisted of a piece of rubber tubing (1 inch, inside diameter) and a pair of intestinal forceps. The other end of the valve ran to another T-tube (J) which was inserted in a 1,500-ml. Erlenmeyer flask (K). The flask (K) provided support for all the equipment inserted in or connected to it. One inch from its bottom, a 3/4-inch tubulature was installed.

Around one end of the T-tube (J), inserted in the supporting flask (K), a polyethylene bag was taped (L). This was used to collect the eructated gas. The dimensions of the bag ($8 \times 4 \times 16$ inches) were such that the entrance of even a small amount of gas could be easily detected, by a slight "puffing out."

Between the supporting flask (K) and the spirometer (N) was a valve (M), exactly the same in construction as valve (I). The spirometer (N) was of the water-seal type. The bell was counter-balanced so that a subatmospheric pressure existed within it. Thus, whenever the valve (M) between the spirometer and the supporting flask (K) was opened, any gas present rushed into the bell. This insured rapid evacuation of the supporting flask (K) and polyethylene bag (L). An escape valve (O) was provided at the base of the spirometer so that the bell could be evacuated when necessary. Although not so shown in Figure 2, the spirometer was calibrated in tenths of a liter, with a total capacity of six liters.

Of the system just described, the part involved with the collection of the eructated gas is a positive one, i.e., the experimental animal must expend a certain amount of energy to force gas into it.

The following procedure was adopted for the measurement of an eructation: *(a)* valve (M) between the collecting system (K and L) and the spirometer (N) was closed after evacuating the collecting system (K and L); (*b*) valve (I) was opened between the collecting system and T-tube (H), whose arms were connected to the mask (A) and the cephalic tracheal cannula (E); *(c)* after an eructation, valve (I) was closed and valve (M) was opened; *(d)* the volume of the belch was noted and step (*a*) repeated, etc.

In order to determine the validity of the method, rumen gas evolution (less that amount absorbed through the rumen wall) was measured by the proposed technique and the already established technique of Cole *et al.* (*6*), where the gas was withdrawn from the rumen through a Wet Test meter by aspiration. The resulting values were compared.

The two animals used in these trials were Holstein steers which had been fitted with permanent rumen cannulae and had transected tracheas. The trials were conducted such that on one day one technique for measuring gas was used and the following day the other technique. Each steer was fed the experimental ration for at least three days before trials were conducted using that particular ration. The rations used were alfalfa hay, oat hay, and fresh alfalfa tops and oat hay. Alfalfa hay and oat hay were fed at the rate of one and one-half pounds per hundred pounds of body weight. One-third of this daily allotment was fed at the time of the trial. Six pounds of alfalfa tops were fed per hundred pounds of body weight with one-fourth of the daily allotment being fed at the trial. The oat hay added to the alfalfa tops diet was fed at the rate of one-half pound per hundred pounds of body weight; the entire amount was fed the night before an alfalfa tops trial.

Where the rumen gas was measured using the eructation technique it was impossible to install the mask while the animal was eating; therefore, data collection began after the animal had consumed his test meal. In the case of the trials where the gas was withdrawn directly from the rumen, measurements were started simultaneously with eating. The latter data were then corrected to correspond to the same time interval covered by the eructation technique.

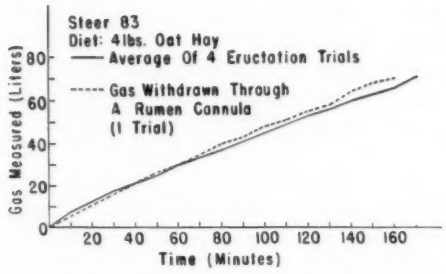

FIG. 4. A comparison of two different methods for measuring rumen gas evolution (less that portion absorbed through the rumen wall). The solid line represents the average of four trials where the eructated gas was measured by a spirometer. The dashed line refers to one trial where the gas was aspirated through a rumen cannula and measured with a Wet Test meter. Steer 83. Diet at trial—four pounds oat hay.

RESULTS

Figure 4 compares the results of trials with Steer 83, where the rumen gas was aspirated directly from the rumen, and trials where the volume of rumen gas was determined according to the eructation technique. Similar results were obtained with another steer and other diets (Table 1).

Regardless of diet or animal, there was a very close agreement between the results of the two methods investigated. Furthermore, the results indicated that the equipment involved with measuring eructated gas did not contribute to the hindrance of gas elimination. If this had been the case, the eructation curve (Figure 4) would be displaced to the right, indicating gas retention.

DISCUSSION

Results show the close agreement between the two different techniques for measuring gas evolved in the rumen. Also, the results in Table 2, which are to be considered in a later paper *(8)*, indicate the close relationship between the two methods for measuring rumen gas. An independent set of data is

TABLE 1

A comparison of two different methods for measuring rumen gas evolution from a steer fed three different rations (less that absorbed through the rumen wall)

	Alfalfa Hay (5 lb.)		Alfalfa tops (15 lb.), oat hay (6 lb.)		Oat hay (5 lb.)	
Time	Eructated	Aspirated	Eructated	Aspirated	Eructated	Aspirated
30	29[a]	22[b]	20[a]	26[b]	22[a]	21[b]
60	52	42	46	63	42	41
90	73	62	83	98	60	56
120	90	78	119	129	78	75
150	107	96	147	152	95	93

The steer used in these trials was No. 802.

[a] Av. of 2 trials.

[b] One trial.

TABLE 2

A comparison of two sets of data on rumen gas measurements

Workers	Diet	Amt. eaten	Trials	Trial length	Gas evolved	Gas per hr.
		(lb.)	*(No.)*	*(hr.)*	*(liter)*	*(liter)*
Cole and[a] Kleiber *(5)*	Alfalfa tops	15.5	4	4	122	30.5
Colvin,[b] Cupps, and Cole *(8)*	Alfalfa tops	14	12	3.3	100	30.3

[a] Gas drawn through a rumen cannula.
[b] Eructated gas measured.

compared with data gathered by means of the eructation technique just described.

According to most workers *(1, 2, 20)*, it is necessary to have a sharp decrease in intraesophageal pressure at the time of normal regurgitation. This is accomplished by the animal, by closing the glottis at the time of a forced inspiratory effort. Simultaneously, the flooded cardiac orifice of the esophagus opens and ingesta is aspirated into the esophagus.

It was shown by Colin *(7)* and Wester *(23)* that regurgitation could occur in tracheotomized cows. On the other hand, Phillipson *(18)* relates that cows with an open trachea regurgitate only with great effort and then only after a period of adjustment to the new conditions.

Our results with regard to regurgitation agreed with the findings of Colin *(7)* and Wester *(23)*. The pattern of regurgitation following the operation continued uninterrupted. The same was true whenever the Edick mask was in place over the animal's muzzle *(16)*.

If the number of chews per bolus can be used as a criterion for a normal-sized bolus, our results with tracheotomized animals are in agreement with those of Schalk and Amadon *(20)*, who used intact animals. On a ration of ground grain, alfalfa silage, and corn silage, they reported that each bolus was chewed an average of 45 times. We found an average of 41 chews per bolus when the diet consisted of oat hay.

There is little doubt that in the intact animal the mechanism of a forced inspiration with a closed glottis is an important, though not absolutely necessary, feature in regurgitation. When the animals with transected tracheas regurgitated, there was a distinct, sharp inspiration which just preceded the passage of the bolus up the esophagus. The effect was clearly audible. When rumen motility records were made according to the technique of Cole *et al. (6)*, this inspiratory effort was indicated by a sharp upward deflection. Though the inspiratory efforts of the experimental animals could not produce the same drop in pressure as in intact animals during regurgitation, some decrease in intraesophageal pressure was produced *(23)*. It appears that either the relative negativity of the pressure within the esophagus necessary for normal regurgitation may vary within wide limits, or that other mechanisms *(23)* are more important.

While testing the eructated gas–measuring equipment, it was observed that during eructation the majority, if not all, of the gas passed through the cephalic tracheal cannula, rather than through the Edick mask *(16)* as expected (Figure 2). If the cephalic tracheal cannula or the tubing leading from the cannula became clogged, gas was retained in the rumen. In such circumstances, gas released from the rumen during eructation was rapidly reswallowed when it could not pass the blocked region. On the other hand, a plugging of the mask had little or no restraining effect on the flow of belched gas.

It is clear that for gas to escape via the cephalic tracheal cannula, the glottis must be open during eructation. At least this is true for the animal preparations used in these experiments. It is possible that the recurrent laryngeal nerves may have been interrupted during surgery. However, it has been indicated *(21)* that reasonable care in the separation of the trachea from its surrounding tissue should leave the nerves intact. Another factor which would serve to indicate that the recurrent laryngeal nerves were uninjured, was the lack of ingesta draining from the cephalic end of the sectioned trachea during regurgitation.

This was the case in all of the animals except one. In this one exception, draining persisted for only a short time. In addition to this, in the steer which had a cannulated tracheal fistula, gas was noticed leaking from around the cannula at each eructation. The trachea, in this instance, was intact except for a hole cut in the ventral aspect. The possibility of having injured the recurrent laryngeal nerves was negligible here. If the cannula was removed and a small balloon inflated in the trachea anterior to the cannulation site, the balloon was blown posteriorly after each eructation. Gas was passing from the esophagus, through the open glottis and down into the trachea. On the basis of the foregoing, it appears that little or no injury was inflicted by tracheal transection upon the nerves concerned with closure of the glottis.

Dougherty, Meredith, and Barrett *(13)* have related that the glottis is closed an instant before eructation, and remains so until all gas is expelled from the esophagus. These workers observed a slight increase in intrapleural pressure as a result of the closed glottis. It was postulated that the pressure increase aided in the expulsion of gas from the esophagus. By inserting a tracheal cannula, they reduced intrapleural pressure considerably. While not stated, it is presumed that eructation still occurred. On his kymograph records, Wester (*23*) illustrated that eructation is invariably associated with the maximum intra-thoracic pressure developed during expiration.

To assist in clearing up this point of glottis closure, it should not be difficult to withdraw a sample of gas from the trachea of an intact animal following a belch and check it for methane concentration. (If the concentration is higher than that of venous blood, this is reasonable evidence to support the idea that the glottis is open during eructation.) As yet, however, this experiment has not been made.

SUMMARY

A technique, involving tracheal transection, to determine the frequency and amplitude of eructation, was developed with seven steers and two cows. To measure the volume of the eructations, a mask was installed on the animal's muzzle and a cannula was inserted in the anterior end of the transected trachea. Tubing connected the mask and cannula to a spirometer. It was found in this type of preparation that most, if not all, of the eructated gas escaped via the anterior trachea. The volume of gas evolved in the rumen (less that absorbed through the rumen wall) was measured in ten trials with the eructation technique. These values compared favorably with those recorded from four trials using a method where the gas was aspirated directly from the rumen. For the comparison, two steers and diets of alfalfa hay, oat hay, and alfalfa tops–oat hay were employed. The rumination reflex appeared to be unimpaired.

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CAROTENOID AND VITAMIN A CONTENT OF MILK AND BLOOD OF DAIRY CATTLE FED MODERATE AMOUNTS OF GROUND SOYBEANS, SOYBEAN OIL MEAL, AND LINSEED OIL MEAL

C. F. MONROE,[1] J. W. HIBBS, AND R. G. WASHBURN
Ohio Agricultural Experiment Station, Wooster

Total milk production and carotenoids and Vitamin A in the milk of cows and in the blood of heifers fed liberal amounts of roughage were not affected by soybeans fed with a simple grain ration. *Editor.*

As early as 1933, Hilton *et al. (7, 8)* observed that late-cut soybean hay when fed to dairy cows resulted in lower vitamin A activity in the butterfat than would be expected from the carotenoid intake from the hay. Wilbur *et al. (15)* showed that this vitamin A–suppressing factor of late-cut soybean hay was associated with the beans. In vitro observations of carotene destruction, when an oily solution of carotene was mixed with a suspension of uncooked ground soybeans, were made in 1936 by Frey *et al. (4)*. Hauge *et al. (5)* later demonstrated that this factor was present in both soybean oil and soybean oil meal and that it was not readily extracted by the expeller process or by chemical solvents, including ethyl ether and ethyl alcohol. Later, they *(6)* were able to adsorb the factor on "Nuchar," an activated carbon, and found that it was not eluted from the adsorbent by acetone, although the acetone did extract the oil, sterol glucosides, coloring matter, and other substances from the column. A special aluminum silicate failed to adsorb the factor.

Subsequent experiments conducted by Shaw *et al. (12, 13)* and Squibb *et al. (14)* have further demonstrated the vitamin A–suppressing action of soybeans in the blood of both cows and calves. Both carotene and vitamin A have been affected to varying degrees in different experiments in which relatively large amounts of soybeans were included in the ration. Ellmore and Shaw have indicated *(3)* that at least 64 micrograms of carotene per pound of body weight per day were required to maintain a safe margin in calves fed a ration containing 30% soybeans, either cooked or raw.

In the three experiments to be reported here, the primary aim was to determine whether or not carotene would be destroyed or its absorption or conversion to vitamin A would be interfered with, using milk and blood carotenoids and vitamin A levels as criteria, if ground soybeans were fed in amounts ordi-

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[1] Retired in 1951, now deceased.

narily used to supply the protein in a simple grain mixture fed with liberal roughage intake. This information would be of practical value to dairymen who might wish to feed raw soybeans under certain economic conditions. Although of secondary importance in this study, average milk production and butterfat per cent of the cows in the different ration groups are recorded, from which over-all ration effects can be assessed. Brief summaries of this work previously were made by Monroe *et al. (10, 11)*.

EXPERIMENTAL PROCEDURE AND RESULTS

Trial 1. Ground soybean vs. soybean oil meal—lactating cows. In the first experiment two grain mixtures were compared at the Trumbull County Experiment Farm in a continuous feeding trial involving a total of 28 Holstein cows and 51 lactations. One grain mixture consisted of 470 lb. ground corn, 300 lb. oats, 230 lb. ground soybeans, 10 lb. iodized salt, and 10 lb. bonemeal (approximately 15.0% crude protein, 6.8% fat). The other grain mixture contained 500 lb. ground corn, 300 lb. oats, 200 lb. soybean oil meal, 10 lb. iodized salt, and 10 lb. bonemeal (approximately 15.4% crude protein, 3.8% fat).

One of these two grain mixtures was fed to one-half of the cows and the other mixture to the other one-half, throughout the lactation, and to some cows for as many as three lactations during the course of the experiment. Grain feeding was limited to approximately one lb. to five lb. of milk produced. The roughage consisted of mixed grass legume hay and corn silage, fed in liberal amounts, and pasture in season.

In order to measure the possible effect of the ground soybeans on the vitamin A and carotene concentration of the milk, composite samples of milk from the cows on experiment were analyzed for both carotenoids and vitamin A (γ/100 ml. whole milk) on four different dates during the two-year period. The method used was that described by Boyer *et al. (2)*, substituting a 10 min. hot saponification for the cold saponification. Micrograms of vitamin A and carotenoids per gm. of butterfat were calculated by dividing the γ/100 ml. by butterfat per cent. Total vitamin A potency was calculated as follows: U.S.P. units/100 ml. =

$$\frac{\text{Carotenoids } (\gamma/100 \text{ ml.})}{0.6} \text{ plus vitamin A } (\gamma/100 \text{ ml.}) \times 4.$$

Data in Table 1 show that while the carotenoids and vitamin A content of the milk varied on the different dates, no depressing effect resulted from the ration containing ground soybeans.

Average milk production was not greatly different in either group. Cows fed the grain ration containing ground soybeans produced 11,322 lb. of milk (11,050 lb. 4% F.C.M.) in 320 days, compared to 12,000 lb. (10,665 lb. 4% F.C.M.) by the group fed the grain ration containing soybean oil meal. Butterfat production averaged, respectively, 409 lb. and 417 lb. Average butterfat per cent in the soybean group was 3.61 and in the soybean oil meal group, 3.47.

Trial 2. Linseed oil meal vs. soybean oil meal vs. ground soybeans—lactating cows.

TABLE 1

Carotenoid and vitamin A content of milk sampled from Holstein cows fed either soybean oil meal or ground soybeans as the protein supplement in a simple grain mixture with liberal roughage—Trial 1

Date milk sampled	Protein supplement	Carotenoids		Vitamin A		Vitamin A activity	
		Milk	Butterfat	Milk	Butterfat	Milk	Butterfat
		(γ/100 ml.)	(γ/g[1])	(γ/100 ml.)	(γ/g[1])	(U.S.P. units/100 ml.)	(U.S.P. units/g)
November 19, 1948	Soybean Oil Meal	18.2	5.25	20.9	6.13	113.9	33.25
	Ground Soybeans	14.8	4.10	20.5	5.82	106.7	30.11
March 3, 1949	Soybean Oil Meal	16.2	4.67	13.6	3.92	81.4	23.46
	Ground Soybeans	27.4	7.59	16.7	4.63	112.5	31.17
April 5, 1950	Soybean Oil Meal	10.6	3.11	13.8	3.97	72.9	21.06
	Ground Soybeans	10.3	2.85	13.1	3.63	69.6	19.27
May 11, 1950	Soybean Oil Meal	9.4	2.71	15.4	4.44	77.3	22.28
	Ground Soybeans	9.5	2.63	18.0	4.99	87.8	24.34
Av. (4 dates)	Soybean Oil Meal	13.6	3.94	15.9	4.62	86.4	25.05
	Ground Soybeans	15.5	4.29	17.1	4.77	94.2	26.23

[1] Butterfat per cent was not determined for these composite samples. Average butterfat per cent throughout the trial based on monthly analyses was 3.47% and 3.61% in the soybean oil meal and the ground soybean groups, respectively. Micrograms/g of fat was calculated from micrograms/100 ml. of whole milk, using these average butterfat percentages.

In Trial 2, three groups of five cows, each consisting of three Holsteins and two Jerseys, were used. This experiment was conducted at the Ohio Agricultural Experiment Station, Wooster. Carotenoids and vitamin A content (γ/100 ml.) and butterfat per cent were determined in the milk of the individual cows to be used in the experiment (A.M. and P.M. composite samples) on two days, two weeks apart, during a pre-experimental period in which all cows were fed the same grain mixture and were adjusted to the same amounts of alfalfa hay and corn silage to be fed in the experimental period.

At the beginning of the seven-week experimental period each of the three groups was assigned to be fed either one of the grain mixtures containing ground soybeans or soybean oil meal, described in Trial 1, or a third grain mixture containing 405 lb. ground corn, 300 lb. oats, 250 lb. linseed oil meal, 10 lb. salt, and 10 lb. bonemeal (approximately 15.3% crude protein, 4.0% fat). Corn silage (approximately 29.5% dry matter—6.8 γ/gm carotenoids) was fed at the rate of 36 lb. per day to Holsteins and 30 lb. per day to Jerseys, and alfalfa hay of medium quality (approximately 9.1% protein and 11.0 γ/gm carotenoids) was fed free choice. Average intake of hay was 16.4 lb. per day for Holsteins and 8.4 lb. per day for Jerseys. Average daily amount of grain fed was 8.7 lb. to Jerseys and 11.6 lb. to Holsteins.

Individual A.M. and P.M. composite milk samples were analyzed for carotenoids and vitamin A content (γ per 100 ml.) and butterfat per cent at the end of three, six, and seven weeks after the beginning of the experimental feeding period. Results of the carotenoid and vitamin A analyses obtained in both the preliminary and experimental periods are shown (Table 2) on a group average basis, along with micrograms of carotenoids and vitamin A per gram of butterfat

TABLE 2

Average carotenoid and vitamin A content of milk from two Jersey and three Holstein cows fed either linseed oil meal, soybean oil meal, or ground soybeans as the protein supplement in a simple grain mixture fed with liberal roughage—Trial 2

Date milk sampled	Protein supplement	Carotenoids		Vitamin A		Vitamin A activity	
		Milk	Butterfat	Milk	Butterfat	Milk	Butterfat
		(γ/100 ml.)	(γ/g)	(γ/100 ml.)	(γ/g)	(*U.S.P. units* 100 ml.)	(*U.S.P. units*/g)
November 28, 1950 (prelim.)	Soybean Oil Meal	26.7	6.17	14.9	3.93	104.2	25.99
	Soybean Oil Meal	30.6	5.97	16.9	3.63	118.5	24.48
	Soybean Oil Meal	24.8	5.71	16.2	4.00	106.3	25.54
December 12, 1950 (prelim.)	Soybean Oil Meal	24.0	5.72	21.8	5.74	126.7	32.36
	Soybean Oil Meal	27.9	5.55	23.3	5.26	139.6	30.28
	Soybean Oil Meal	20.7	5.02	22.0	5.69	122.6	31.14
January 10, 1951[1] (after three wk. on expt.)	Linseed Oil Meal	16.4	4.43	16.0	4.98	91.5	27.33
	Soybean Oil Meal	22.0	4.64	15.8	3.87	100.6	23.42
	Ground Soybeans	16.9	4.08	17.9	4.45	99.7	24.75
January 22, 1951 (after five wk. on expt.)	Linseed Oil Meal	14.8	4.05	16.6	5.10	91.2	27.15
	Soybean Oil Meal	19.9	3.94	16.6	3.87	99.8	22.03
	Ground Soybeans	14.6	3.46	18.2	4.58	97.3	24.08
February 5, 1951 (after seven wk. on expt.)	Linseed Oil Meal	13.2	3.96	11.6	3.57	68.3	20.42
	Soybean Oil Meal	17.6	3.72	14.7	3.58	88.3	20.52
	Ground Soybeans	13.1	3.16	14.7	3.74	80.4	20.03

[1] Experimental feeding period began December 21, 1951.

and U.S.P. units of vitamin A activity per 100 ml. of milk, which were calculated as previously described.

During the three-week preliminary period, the average daily production of the five-cow groups that were subsequently assigned, respectively, to the linseed oil meal, soybean oil meal, and ground soybean groups was 34.2, 36.4, and 35.6 lb. Four per cent F.C.M. averaged, respectively, 32.4, 37.6, and 35.3 lb. Average daily butterfat production was, respectively, 1.25, 1.54, and 1.35 lb. The average butterfat per cent for the preliminary period in the three groups was, respectively, 3.65, 4.21, and 3.79.

During the experimental period daily milk production for the same three groups averaged, respectively, 30.4, 34.0, and 31.4 lb. Four per cent F.C.M. averaged, respectively, 27.8, 34.2, and 31.3 lb. Daily butterfat production averaged, respectively, 1.05, 1.37, and 1.25 lb. Average butterfat per cent for the period was, respectively, 3.43, 4.01, and 3.99. The slightly greater over-all drop in milk production in the group fed ground soybeans, compared to the other two groups, was largely due to a temporary decline during the third and fourth weeks of the experimental period. During the last three weeks of the experimental period, milk production of this group returned to the expected level, based on the production level during the preliminary period and the early part of the experimental period.

Trial 3. Linseed oil meal vs. ground soybeans—heifers. In this experiment, which was also carried out at Wooster, eight Jersey heifers, average weight 670 lb., were divided into two groups. One group was fed mixed alfalfa-timothy hay (approximately 9.5% protein and 12.5 micrograms/gm. carotenoids) free

choice with six lb. of the grain mixture containing linseed oil meal, described earlier. The other group was fed the same hay and six lb. daily of the previously described grain mixture containing ground soybeans. After 10 weeks the hay was changed to rather poor quality mixed clover-timothy hay (approximately 7.2% crude protein and 3.5 microgram/gm. carotenoids). The experiment was then continued, using the same grain feeding schedule for an additional six weeks.

Twice during the two-week preliminary period and approximately at weekly intervals during the experimental period, jugular blood samples were taken for plasma carotenoid and vitamin A analysis. The method of Kimble *(9)* was followed except that when the plasma carotenoids exceeded 300 micrograms per 100 ml. they were removed prior to vitamin A analysis, using the technique described by Boyer *et al. (1)*.

Average weekly values for plasma carotenoids and vitamin A of the four heifers in each group are shown graphically (Fig. 1). Also indicated at each bleeding date is the average weekly hay consumption of the groups for the preceding week.

DISCUSSION

While fluctuations did occur in the carotene and vitamin A content of the milk (Tables 1 and 2) and blood of the heifers (Figure 1) during the experimental periods, they were attributed to factors other than the influence of the

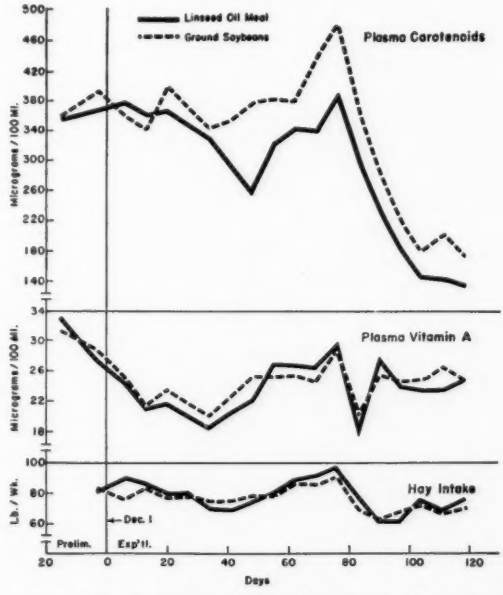

FIG. 1. Changes in the blood plasma carotenoids and vitamin A, and variations in hay consumption, of heifers fed either linseed oil meal or ground soybeans, Trial 3. Poor quality hay was fed after the 70th day of the experimental period.

ground soybeans. Among the factors that may have caused these fluctuations were variations in the carotene content of the roughage, variations in roughage consumption, and seasonal effects associated with carotene intake which result in physiological blood level changes involving mobilization of liver vitamin A.

Estimated carotenoid intakes of the cows in Trial 2 and the heifers in Trial 3, during the time good hay was fed, were well above the 64 γ/lb/day level of carotene which was found by Ellmore and Shaw *(3)* to be required to maintain a margin of safety in calves fed a ration containing 30% soybeans. During the period in which the heifers in Trial 3 were fed poor hay, an estimated carotenoid intake of only 21 γ/lb/day resulted. In this period both groups declined rapidly in blood carotenoids, but the group fed ground soybeans declined no faster than the group fed linseed oil meal. It is possible that some differences might have resulted had the poor hay feeding been prolonged. The significance of these observations is even more pertinent, when it is considered that the *beta* carotene in the total carotenoids consumed was only about one-half the total, and that the actual vitamin A activity of the total carotenoids was much less than total carotenoids might indicate.

In none of these experiments was there any measured evidence that ground soybeans, fed under these practical conditions, caused destruction of carotene or interfered with its absorption or conversion to vitamin A.

CONCLUSION

Analyses of the carotenoids and vitamin A in the milk of cows and in the blood of growing heifers fed liberal amounts of good roughage showed no evidence that ground soybeans caused carotene destruction or interfered with its absorption or conversion to vitamin A when fed as the protein supplement in simple grain rations. Therefore, under practical dairy feeding conditions, where good roughage is fed liberally, soybeans could be used, where advantageous, as the protein supplement in grain mixtures without danger of vitamin A deficiency. No significant effects of feeding ground soybeans on total milk production were observed in these experiments. Butterfat per cent was higher during the periods when ground soybeans were fed.

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EFFECT OF GLYCEROL EQUILIBRATION ON FROZEN BOVINE SPERMATOZOA[1]

E. F. GRAHAM, W. E. ERICKSON, AND N. D. BAYLEY[2]
Department of Dairy Husbandry, University of Minnesota, St. Paul

Semen was collected from six bulls, to determine the effects of glycerol equilibration time before freezing on fertility of the spermatozoa. Fertility during three weeks of storage was not affected, but there was a higher percentage nonreturn rate for each bull for the longer equilibration. *Editor.*

Considerable attention has been given to the determination of optimum conditions for freezing bovine spermatozoa. To utilize the generally practiced morning routine of semen collection for building frozen semen banks, and working the freezing procedure into a normal day, much interest has been shown in decreasing the length of equilibration time. For this purpose, and for the purpose of trying to establish the optimum length of glycerol equilibration time, this study was undertaken.

Little information based on fertility trials is available on the optimum length of equilibration for any one set of conditions. Polge and Rowson *(12)* indicate that optimum recovery and fertility are obtained with from 15 to 20 hours of glycerol equilibration. Stower *(14)* and Holt *(8)* reported best results with equilibration of from 20 to 24 hours. Williams *(15)*, however, reports a higher percentage of nonreturns when cows are inseminated with semen equilibrated six hours, as compared to that equilibrated 18 hours. Emmens and Blackshaw *(4)* have also obtained satisfactory results with shorter equilibration periods. Several investigators used microscopic determination of percentage of progressively motile spermatozoa in establishing the optimal length of glycerol equilibration time.

Saroff and Mixner *(13)* state that as glycerol equilibration time is increased from two to 18 hours, there is a progressive increase in sperm survival on thawing, after freezing to —75° C. Cragle and Myers *(2)*, in a series of experiments, indicated that acceptable recovery of progressively motile spermatozoa could be attained if allowed to equilibrate in the presence of glycerol for 6.5 to 16 hours. They also found that decreasing the equilibrating time to four hours, or increasing it to 28 hours, resulted in a lower percentage of recovery of live sperm cells. Later Cragle *et al. (3)*, applying a three-dimensional central composite design and testing for the optimal combination for per cent citrate, per cent

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[1] Paper No. 3576, Scientific Journal Series, Minnesota Agricultural Experiment Station.

[2] Present address: Dairy Husbandry Research Branch, USDA Research Service, Beltsville, Maryland.

glycerol, and glycerol equilibration hours, estimated the optimum equilibration time to be 14.9 hours. Miller and Vandemark *(9)* and Bruce *(1)* found six hours of glycerol equilibration adequate for optimum survival of bull spermatozoa. O'Dell and Hurst *(11)* compared the effects of zero-hour, or the least possible, and 18-hour glycerol equilibration. Their results, based upon per cent recovery of sperm cells 48 hours and ten days after freezing, indicated a statistically higher recovery rate when equilibrated in glycerol for zero hour than when equilibrated 18 hours. O'Dell and Almquist *(10)* found no significant difference, comparing 30 minutes, four hours, and 18 hours of glycerol equilibration.

MATERIALS AND METHODS

In this study, semen was collected from six mature Holstein bulls selected at random from locations of six different artificial breeding organizations. Semen was collected from these bulls by the use of an artificial vagina, on their normal weekly schedule date. In most cases, a mixture of the first and second ejaculate was used for freezing. Semen collected from five bulls was frozen in pasteurized, homogenized whole milk that had been heated to +92° C. for five minutes. The final concentration of glycerol used in this extender was ten per cent *(5)*. The semen from one bull was frozen in a yolk-citrate medium containing a final concentration of seven per cent glycerol, 2.37% trisodium citrate (dihydrate), and 23.25% egg yolk. The antibiotics, penicillin (potassium crystalline G) and dihydrostreptomycin sulfate, were incorporated into the extender at a rate of 1,000 units and 1,000 μ per ml., respectively *(5)*.

Immediately after collection, enough semen was added to ten ml. of extender to yield a dilution of 1:40 on final addition of glycerolated buffer. This small amount of extended semen was then allowed to cool to +4° C. and remained at this temperature for three and one-half hours. The glycerolated buffer was then added to the extended semen at ten-minute intervals, in progressively larger quantities. Portions of the extended, glycerolated semen was allowed to equilibrate four, eight, and 12 hours at +4° C., prior to ampouling and freezing. Freezing was done according to the method described by Graham and Marion *(7)*, 1953. Rate of freezing of semen in the milk extender was 2° C. per minute from +5° C. to −30° C. and 5° C. per minute from −30° C. to −79° C. Semen extended in yolk-citrate was frozen at 3° C. per minute from +5° C. to −15° C. and at 5° C. per minute from −15° C. to −79° C. These rates of freezing were based on the results by Erickson *et al. (5)* and Graham *et al. (6)* found (in 1954) to be optimum.

Field trials were conducted in three parts, representing three different semen collection periods from the six different bulls. There were 51 different technicians involved in this trial. Although six to 12 inseminators were used per bull, the same technicians were used for all three of the different collection periods. Two samples of frozen semen of each glycerol equilibration time were sent to each technician after one, two, and three weeks of storage in each period. The technicians selected the semen samples at random from their field-kit and

used them on the first six first-service cows of the breed. The semen samples were stored at —79° C. until used. Samples were thawed at approximately 5°C. After each insemination, the technician wrote down on I.B.M. cards the necessary data to identify the cow, semen sample, date of service, collection period, and week of storage.

Motility estimates were made immediately before and after freezing. Samples of semen frozen under the various conditions were also checked before shipment to the technicians.

RESULTS AND DISCUSSION

A summary of the nonreturn rates for different glycerol equilibration times is given (Table 1). Nonreturn rates were based on 75-day nonreturns, which should be comparable to 60- to 90-day nonreturns.

TABLE 1

Nonreturn rates for the various lengths of glycerol equilibration

12-hr. GEQ [a]			8-hr. GEQ			4-hr. GEQ		
First services	N.R.	N.R. 75 days	First services	N.R.	N.R. 75 days	First services	N.R.	N.R. 75 days
(*No.*)	(*No.*)	(%)	(*No.*)	(*No.*)	(%)	(*No.*)	(*No.*)	(%)
1,012	686	67.8	1,026	670	65.2	996	631	63.4

[a] GEQ = Glycerol Equilibration Time.

Difference in fertility results obtained with 12-hour and four-hour glycerol equilibration was significant at the five per cent level, the χ^2 value being 4.27. The difference between 12-hour and eight-hour or between eight-hour and four-hour glycerol equilibration was not significant.

Table 2 data show how the difference in glycerol equilibration time affected the conception rate for each individual bull.

Difference within bulls on glycerol equilibration times is significant at the five per cent level on only one bull, H–4. This bull shows a significant difference, as measured by χ^2, in nonreturn rate between 12-hour and four-hour

TABLE 2

Effect of glycerol equilibration time on nonreturn for individual bulls

Bull	12-hr. GEQ [a]			8-hr. GEQ			4-hr. GEQ		
	First services	N.R.	N.R. 75 days	First services	N.R.	N.R. 75 days	First services	N.R.	N.R. 75 days
(*No.*)	(*No.*)	(*No.*)	(%)	(*No.*)	(*No.*)	(%)	(*No.*)	(*No.*)	(%)
H–1	135	94	69.6	143	86	60.1	154	99	64.3
H–2	198	130	65.7	209	135	64.6	201	117	58.2
H–3	199	145	72.8	190	136	71.6	198	141	71.2
H–4	111	87	78.4	113	77	68.1	112	76	67.9
H–5	158	100	63.3	159	103	64.8	121	69	57.0
H–6	211	130	61.6	212	133	62.7	210	129	61.4
Total	1,012	686	67.8	1,026	670	65.2	996	631	63.4

[a] GEQ = Glycerol Equilibration Time.

glycerol equilibration. Even though a significant difference between the various glycerol equilibration times was noted on only this one individual bull, the nonreturn rate was higher for all bulls on 12-hour equilibration than on four-hour, and in most cases higher than on eight-hour, equilibration.

The difference between semen collections within bulls is shown (Table 3).

TABLE 3

Nonreturn rates for different semen collections within bulls

Bull	Collection Period 1			Collection Period 2			Collection Period 3		
	First services	N.R.	N.R. 75 days	First services	N.R.	N.R. 75 days	First services	N.R.	N.R. 75 days
(*No.*)	(*No.*)	(*No.*)	(%)	(*No.*)	(*No.*)	(%)	(*No.*)	(*No.*)	(%)
H–1	163	107	65.6	160	110	68.8	109	62	65.9
H–2	209	129	61.7	193	128	66.3	206	125	60.7
H–3	212	153	72.2	182	135	74.2	193	134	69.4
H–4	107	85	79.4	108	76	70.4	121	79	65.3
H–5	186	135	72.6	181	86	47.5	71	51	71.8
H–6	216	134	62.0	203	134	66.0	214	124	57.9
Total	1,093	743	68.0	1,027	669	65.1	914	575	62.9

Although the number of first-service cows bred varied between collection periods, approximately the same number of cows were serviced with semen equilibrated at the various lengths for any one collection period within bulls. As shown (Table 3), considerable variation exists between different collection periods for each bull. From the results as analyzed by χ^2, significant differences are shown between collection periods. The freezability and fertility of frozen semen collected at different times show considerable variation.

Approximately the same number of first-service cows were bred each collection period, with a split-semen sample equilibrated at the three different lengths for three consecutive weeks. The nonreturn rates for the first, second, and third week of storage, and for the different lengths of equilibration, are shown (Table 4).

The difference between one, two, and three weeks of storage (Table 4) was not significant. There was no interaction noted between glycerol equilibration time and storage and fertility rate during the first three weeks.

Correlation between motility rating and conception rate was determined, using the average progressive motility rating and corresponding conception rate

TABLE 4

Nonreturn rates for the various storage-time intervals and effects of varying lengths of glycerol equilibration

Length of equilibration	1-week storage			2-week storage			3-week storage		
	First services	N.R.	N.R. 75 days	First services	N.R.	N.R. 75 days	First services	N.R.	N.R. 75 days
(*hr.*)	(*No.*)	(*No.*)	(%)	(*No.*)	(*No.*)	(%)	(*No.*)	(*No.*)	(%)
12	343	231	67.3	352	242	69.0	317	212	66.9
8	329	220	66.9	355	230	64.8	342	220	64.3
4	355	230	64.8	344	206	59.8	297	195	65.7
Total	1,027	681	66.4	1,051	679	64.6	956	627	65.6

for each equilibration time, weeks of storage, and collection period within each bull. The range in motility ratings was between 35.5 and 60.0%, with an over-all average of 52.8%. The range in fertility data for any of the above possible combinations was 43.8 to 86.1%, with an average of 65.0%, based on 75-day nonreturns. A correlation between 54 weekly average readings and conception rate was 0.261. This correlation is just short of significance at the five per cent level.

SUMMARY

Three separate semen collections were taken from six different bulls, for testing the effects of glycerol equilibration time before freezing on the fertility of frozen bovine spermatozoa. Portions of the separate semen collections from the different bulls were allowed to equilibrate four, eight, and 12 hours prior to freezing. A total of 3,038 cows was bred, by 51 different technicians, of which 1,012 first-service cows were bred with semen equilibrated 12 hours with a nonreturn rate, based on 75-day nonreturns, of 67.8%. With semen equilibrated eight hours, 1,026 first-service cows were bred with a nonreturn rate of 65.2%, and 996 cows were bred with semen equilibrated four hours, with a nonreturn rate of 63.4%. The difference between four- and 12-hour equilibration was just significant at the five per cent level.

There was a significant difference between different lengths of equilibration within bulls for only one bull, with a highly significant difference in favor of longer equilibration. However, in general, for each bull, a higher percentage nonreturn rate was obtained for the longer equilibration.

There was significant difference in nonreturn rates between collection periods within three of the six bulls.

There were no differences between one, two, and three weeks of storage prior to use. Fertility during the three-week storage period was not influenced by the different lengths of glycerol equilibration.

A positive but nonsignificant correlation was found between fertility rate and motility rate as measured by microscopic examination.

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SEASONAL VARIATION IN THE SUCCINIC DEHYDROGENASE ACTIVITY OF BOVINE SPERMATOZOA

J. T. SMITH, D. T. MAYER,[1] AND C. P. MERILAN

Departments of Agricultural Chemistry,[2] Animal Husbandry, and Dairy Husbandry
University of Missouri, Columbia

The succinic dehydrogenase activity of bovine spermatozoa was increased by washing during the spring and summer but not during the winter. *Editor.*

Seasonal variation in semen quality was first reported by Mills and Senior *(10)*, who were investigating the effect of climate upon human conception rate. Hotchkiss *(5)* could not establish any definite trend in the variation of total spermatozoan counts, but he did observe that in the month of April the semen specimens from a series of healthy men had the highest spermatozoan concentration.

In the case of the rodent, complete cessation of spermatogenic activity was observed during the summer months by Wells *(17)*, and a similar phenomenon was demonstrated in sheep by McKenzie and Berliner *(8)*.

In dairy bulls, Weatherby *et al. (15)* found a decrease in the volume of the ejaculate with season. Anderson *(1)* obtained both a lowered spermatozoan motility and mean semen volume in May, June, July, and August; whereas, Erb *et al. (3)* found the average concentration and the total number of spermatozoa to be at a maximum during April, May, and June. Salisbury *(12)* found the lowest average spermatozoan concentration count during August. A lower level of fertility during June and July, accompanied by a low level of spermatogenesis, was reported by Mercier and Salisbury *(9)*. Weeth and Herman *(16)* demonstrated that the most consistent seasonal trend was a maximum spermatozoan longevity in June and July in semen from Holstein, Jersey, and Guernsey bulls; then a sharp decline to a minimum in September, with a significant increase in October. Lasley and Bogart *(6)* observed a seasonal variation in the semen of the range beef-bull.

It is the purpose of this paper to report the results of an investigation of the effect of season upon the dehydrogenase activity of bovine spermatozoa. Pooled samples from several bulls were used, since cholesterol, DPN, and other analyses also were made on these samples.

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MATERIALS AND METHODS

The semen was collected by use of the artificial vagina from healthy dairy bulls maintained as a regular part of the University of Missouri Agricultural Experiment Station's dairy herd. The vial of semen was placed immediately in a thermos bottle of water at a temperature of 15° C., for transport to the laboratory. When laboratory storage was desired, the vial of semen was placed in a 400-ml. beaker of water and placed in the refrigerator; the large volume of water insured gradual cooling to a storage temperature of 4° C. and the prevention of cold shock.

Semen from two or more bulls was pooled and divided into two portions. The semen was obtained from the same bulls throughout the experimental period. One portion provided the unwashed spermatozoa. The second portion was freed of seminal plasma by centrifugation and spermatozoa were then washed four times with isotonic saline solution. Sufficient saline solution was then added to the washed cells to reconstitute the original semen volume of this portion.

Average spermatozoan concentration was obtained as described by Smith and Mayer *(13)*.

Since spermatozoan suspensions are difficult to prepare with sufficient optical transparency to facilitate application of spectrophotometric techniques for the determination of dehydrogenase activity, the manometric method of Quastel and Wheatley *(11)* was employed to estimate the relative enzymatic activity.

Mean monthly temperatures were obtained from the U. S. Weather Bureau at Columbia, Missouri.

RESULTS

Since the data (Table 1) had suggested that the variation in activity of the succinic dehydrogenase of bovine spermatozoa might be seasonal, the correlation between the mean monthly temperatures and the mean monthly succinic dehydrogenase activity of both washed and unwashed bovine spermatozoa was investigated. A regression line of $Y = 46.4 - 0.464X$ was obtained for the relationship between the mean monthly succinic dehydrogenase activity of bovine spermatozoa and mean monthly environmental temperatures, with a coefficient of correlation of -0.731 ($P = <0.01$). For unwashed spermatozoa a regression line of $Y=37.6 -0.246X$ was obtained for this relationship, with a coefficient of correlation of -0.765 ($P = <0.01$).

Seasonal variations in enzyme activity, both in washed and unwashed spermatozoa, are shown (Table 2). These data, in addition, demonstrate the marked effect of washing upon the dehydrogenase activity of the spermatozoa. Because the regular seasons officially change during a particular month, these data have been taken for the whole months and do not, therefore, represent the true seasons as determined by the relative position of the earth and the sun; for this reason, the particular months involved are cited in Table 2. These data show that during the spring and winter months, washing of spermatozoa resulted in

TABLE 1

Seasonal variation in the succinic dehydrogenase activity of washed and unwashed bovine spermatozoa

Month	Washed[a]	Unwashed[a]	Mean temperature
3/54	61.9	41.3	41.2
5/54	39.4	37.6	59.9
6/54	16.8	25.9	77.2
7/54	27.3	23.1	85.4
8/54	17.0	25.7	79.4
9/54	17.0	27.1	73.6
10/54	12.6	30.2	58.0
12/54	43.3	38.4	46.0
12/54	41.1	36.1	35.5
1/55	36.5	37.7	31.7
2/55	49.1	31.6	32.9
3/55	39.0	39.3	43.5

Regression line washed $Y = 46.4 - 0.464X$; $r = -0.731$ $P = < 0.01$.
Regression line unwashed $Y = 37.6 - 0.245X$; $r = -0.756$ $P = < 0.01$.
Conditions: Washed spermatozoa were washed four times with isotonic saline and resuspended in sufficient saline to re-attain the original seminal volume; unwashed, used as collected.
Flask Contents: $NaHCO_3$, 1.86×10^{-2} M; Na-succinate, 0.08 M; $K_3Fe(CN)_6$, 0.2 ml. of an 11% solution neutralized with $NaHCO_3$; 0.5 and 0.3 ml. of spermatozoan suspension; sufficient saline to make the total flask volume 2.5 ml.
Gas Phase: 5% CO_2–95% N_2, Temperature 38° C. Incubation time, one hour.
Results are expressed as mm^3CO_2 liberated by 10^8 spermatozoa per hour; temperatures in degrees Fahrenheit.

[a] Each figure representing the dehydrogenase activity is the mean of six to eight determinations.

TABLE 2

Seasonal variation in the effect of washing upon the succinic dehydrogenase activity of bovine spermatozoa

Season	Month	Washed	Unwashed	Spermatozoan concentration mm^3
Spring[a]	March, April, May	56.2	38.6	976,000
Summer	June, July, August	19.3	17.0	1,185,000
Fall	September, October, November	23.3	28.4	939,000
Winter[b]	December, January, February	43.8	37.4	962,000

[a] = < 0.01.
[b] = < 0.05.
Conditions same as for Table 1; results expressed as mm^3CO_2 liberated by 10^8 spermatozoa per hour.

a significant apparent activation of their succinic dehydrogenase activity. A slight, though not statistically significant, apparent stimulation of succinic dehydrogenase activity of bovine spermatozoa was obtained during the summer months. In contrast, the effect of washing upon the succinic dehydrogenase in the fall months was not statistically significant.

DISCUSSION

The experimental finding of a definite seasonal variation in the dehydrogenase activity of bovine spermatozoa, particularly succinic dehydrogenase,

would seem to be primarily of fundamental rather than practical importance. However, much of the data reported in the literature on spermatozoan metabolic activities has been based on relatively few determinations. Data obtained in this investigation would suggest that conclusions drawn from a limited sampling of semen specimens, without consideration of environmental temperature or season, would be contradictory. Further, the results reported herein suggest that the data should be interpreted with due consideration of season and of treatment prior to the determination of metabolic rate.

Establishment of a seasonal variation in the succinic dehydrogenase activity of bovine spermatozoa is of importance, also, because it identifies a definite metabolic system which has been affected by environmental temperature changes. Previously, it has been shown that variation in spermatozoan concentration, per cent abnormals, longevity, ejaculate volume, and per cent motile spermatozoa, were in some way altered by seasonal temperature fluctuations.

Although the importance of an active succinic dehydrogenase to the normal metabolic activities of bovine spermatozoa has been questioned by MacLeod *(7)*, the importance of the dehydrogenases to spermatozoan activities, in general, has been established by the correlation between certain reduction tests and the per cent nonreturns by Beck and Salisbury *(2)* and by Erb and Ehlers *(4)*. Establishment of an impaired succinic dehydrogenase activity in this investigation, which coincides with the seasons of impaired fertility as previously reported by Mercier and Salisbury *(9)*, would seem to lend increased importance to the application of the reduction tests as a measure of spermatozoan quality, and may indicate that an active succinic dehydrogenase is of importance to spermatozoan viability and fertility.

Data regarding the effect of coenzymes, as well as cholesterol and carotene, upon the succinic dehydrogenase activity of bovine spermatozoa reported by Smith *et al. (14)*, make it appear that it may be a variation in these compounds which is responsible for the observed seasonal variation. However, data demonstrating a seasonal variation in the concentration of any of these compounds in seminal plasma or spermatozoa are not available.

SUMMARY

The effect of season upon the succinic dehydrogenase activity of bovine spermatozoa has been investigated by a manometric method. Succinic dehydrogenase activity of bovine spermatozoa has been found to vary in a manner which was significantly correlated with the mean monthly temperatures as obtained from the local weather bureau. The effect of washing upon the succinic dehydrogenase activity of bull spermatozoa was found to be seasonally dependent, as well. These observations have been discussed in relation to their importance to research in the physiology of reproduction, and to the application of reduction tests as a measure of semen quality.

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EFFECT OF WASHING UPON THE DEHYDROGENASE ACTIVITY OF BOVINE SPERMATOZOA

J. T. SMITH, D. T. MAYER,[1] AND C. P. MERILAN
Departments of Agricultural Chemistry,[2] Animal Husbandry, and Dairy Husbandry, University of Missouri, Columbia

Washing bovine spermatozoa with isotonic saline solution stimulated succinic dehydrogenase activity, but inhibited glyceraldehyde-3-phosphate dehydrogenase activity. *Editor.*

The term "dilution effect" was used by Gray *(3)* to describe an increase in both the motility and respiration of sea urchin spermatozoa upon dilution with sea water.

In its application to mammalian spermatozoa, however, the term dilution effect describes a different phenomenon. Milovanov *(14)*, Salisbury *et al. (16)*, Kennedy *(8)*, Emmens and Swyer *(2)*, and Blackshaw *(1)* have investigated the dilution effect in human, rabbit, bull, and ram spermatozoa, and have observed, in general, that a decrease in longevity and motility accompanies excessive dilution of mammalian spermatozoa.

Effect of washing upon the metabolism of spermatozoa has been investigated by Mann and Lutwak-Mann *(13)* and by White *(22-25)*. Mann and Lutwak-Mann *(13)* observed a decrease in respiration and in fructolysis following excessive dilution of spermatozoa. White *(22-25)* found that washing once with a Na-PO_4-fructose diluent had no effect on motility, but that washing the spermatozoa twice adversely affected motility and lactic acid production. He also established that a certain amount of the washing inhibition is reversible upon the addition of the alkali metals, Rb^+ and K^+. Since K^+ functions in the glycolytic cycle as an aid in the transfer of high energy phosphate from phospho-enolpyruvic acid to ADP, White concluded that it is this system, rather than the oxidative, which is affected by washing of the spermatozoa. He also stated that washing and dilution are similar in their effects upon spermatozoan metabolism.

It is a generally accepted concept that certain dehydrogenases and their coenzymes are soluble and may be removed by extensive washing of a tissue. That dehydrogenases and/or their coenzymes might be removed by the washing of spermatozoa would seem to be an attractive postulate. It is the purpose of

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this paper to report data obtained during an investigation of the effect of washing upon the dehydrogenase activity of bovine spermatozoa, and to discuss the importance of the observed effects to certain aspects of reproductive physiology.

MATERIALS AND METHODS

The semen was collected from healthy dairy bulls maintained as a regular part of the Missouri Experiment Station Dairy herd. An artificial vagina was used to collect the semen, which was immediately placed in a thermos bottle of water at a temperature near 15°C., for transport to the laboratory. When received at the laboratory, the vial of semen was placed in a 400-ml. beaker of water and stored in the refrigerator.

Usually, semen specimens from two or more bulls were pooled and washed with five volumes of isotonic saline. Saline solution and spermatozoa were separated by centrifugation at 2,200 r.p.m. (950 g.) in a Serval Model G Centrifuge. Separation was usually complete after 10 minutes of centrifugation, even when a large proportion of the supernatant was seminal plasma; less time was required when only spermatozoa and saline solution were present. The supernatant was removed by a hand-controlled automatic pipette. Resuspension of the spermatozoa was accomplished by breaking the centrifuge cake with a small stirring rod and repeatedly inverting the tube. Washing was repeated four times, after which the washed sample was resuspended in sufficient isotonic saline to re-attain the original semen volume.

Spermatozoan concentration counts were made, using an eosin counting fluid as described by Smith and Mayer *(18)*. Dehydrogenase activity was estimated by the method of Quastel and Wheatley *(15)*, and aldolase activity determined by the method of Sibley and Lehninger *(17)*.

RESULTS

The washing treatment utilized in this investigation altered the activity of four different spermatozoan metabolic enzymes (see Table 1). The succinic dehydrogenase was most markedly affected. A highly significant stimulation of this enzyme was observed. In contrast, glyceraldehyde-3-phosphate dehydrogenase activity was inhibited significantly. The activity of malic dehydrogenase and aldolase was apparently stimulated, although the enzyme activity of the treated cells did not differ from that of the untreated cells to a statistically significant degree.

A significant DPN (diphosphopyridinenucleotide) inhibition of the spermatozoan succinic dehydrogenase activity was demonstrated by measuring the relative activity of the latter in the presence and absence of added DPN (Table 2). Removal of DPN by washing the spermatozoa is demonstrated by the data presented (Table 2). These data further show that added DPN does not influence the activity either of malic or glyceraldehyde-3-phosphate dehydrogenase in unwashed spermatozoa, in contrast to its stimulatory effect upon washed cells.

TABLE 1

Effect of washing upon the enzyme activity of bovine spermatozoa

Enzyme system	mm^3CO_2 liberated by 10^8 spermatozoa per hour	
	Washed	Unwashed
Succinic dehydrogenase [a]	35.9 (67)	29.1
Malic dehydrogenase [c]	25.0 (31)	21.5
Glyceraldehyde-3-phosphate dehydrogenase [b]	6.6 (12)	10.4
Aldolase	33.0 (9)	29.8

Values in parentheses indicate number of runs which were averaged.

Conditions: All washed spermatozoa were washed four times with isotonic saline and resuspended in sufficient saline to re-attain the original seminal volume.

Flask contents: For all systems except aldolase assay, which was run according to the method of Sibley and Lehninger *(17)*: $NaHCO_3$, 1.86×10^{-2}M; $K_3Fe(CN)_6$, 0.2 ml. of an 11% solution neutralized with $NaHCO_3$; 0.3 and 0.5 ml. of spermatozoan suspension; sufficient saline to make the total flask volume 2.5 ml.

Added when measuring: (1) (*Succinic Dehydrogenase*) : Na-succinate 0.08 M; (2) (*When measuring Glyceraldehyde-3-Phosphate Dehydrogenase)* : DPN 3×10^{-4} M., Nicotinamide 0.03 M., K_2PO_4 1.6×10^{-3} M., KF 1×10^{-2} M., Fructose 1-6 Di-phosphate 6×10^{-3} M.; (3) (*When measuring Malic Dehydrogenase*): DPN 3×10^{-4} M., Nicotinamide 0.03 M., Na-l-malate, 0.1 M., NaCN, 0.2 ml. of a solution of 0.65 neutralized with HCl.

Gas phase: 5% CO_2-95% N_2, Incubation Temp. 38°C., and incubation time one hour. Values are expressed as mm^3CO_2 liberated per 10^8 spermatozoa per hour, after correction for a blank containing no substrate.

[a] $P = <0.01$.
[b] $P = <0.05$.
[c] $P = <0.3 <0.2$.

TABLE 2

Effect of DPN upon bovine spermatozoan dehydrogenase

Enzyme system	mm^3CO_2 liberated by 10^8 spermatozoa per hour	
	No DPN added	3×10^{-4}m. DPN added
Malic dehydrogenase [a]	9.5 (5)	12.9
Malic dehydrogenase [b]	20.2 (4)	18.0
Glyceraldehyde-3-phosphate dehydrogenase [a]	4.8 (2)	8.1
Succinic dehydrogenase [*, a]	18.6 (15)	12.9

[a] Washed spermatozoa.
[b] Unwashed spermatozoa.
[*] $P = <0.01$.
Values in parentheses indicate number averaged.
Conditions and flask contents same as in Table 1.

The stimulatory effect obtained with washed cells is apparently the result of the removal of DPN from the spermatozoa. Keilin and Hartree *(7)* reported a DPN inhibition of succinic dehydrogenase in muscle preparations by DPN catalysis of the reaction malate⟶oxaloacetate. Oxaloacetate has been shown to inhibit succinic dehydrogenase in concentrations as low as $10^{-4}M$. They reported that it was possible to remove the DPN inhibition by adding *l*-glutamate to the incubation mixtures, which removes the oxaloacetate by transamination. In order to ascertain whether DPN removal by washing of bull spermatozoa caused the stimulatory effect upon succinic dehydrogenase activity, *l*-glutamate was added to the spermatozoan medium. Although DPN alone inhibits succinic dehydrogenase activity (Table 2), addition of the *l*-glutamate to the DPN-

TABLE 3

Effect of addition of enzyme-influencing compounds upon the succinic dehydrogenase activity of bovine spermatozoa

Substance added	mm^3CO_2 liberated by 10^8 spermatozoa per hour	
	No addition	Addition
l-Glutamate (2) and *DPN* 3×10^{-4} *M.*	13.5 (10)	15.8
$CaCl_2$ 4×10^{-4}M.[b]	24.13 (12)	24.94
$CaCl_2$ 4×10^{-4}M.[a]	12.4 (3)	11.4
Nicotinamide 0.03 M.[a, b] and $CaCl_2$ 4×10^{-4}M.	24.9 (12)	21.8
Nicotinamide 0.03 M.[a] *DPN* 3×10^{-4}*M.*, and $CaCl_2$ 4×10^{-4}M.	12.4 (11)	12.6

[a] = Washed spermatozoa.
[b] = Unwashed spermatozoa.
* $P = < 0.01$.
Values in parentheses indicate number averaged.
Conditions and flask contents are the same as in Table 1.
Italicized compounds were added to all flasks; unitalicized compounds, only variable.

containing medium resulted in a slight stimulation of the dehydrogenase activity (shown Table 3).

Mann *(12)* observed a DPNase in both seminal plasma and washed spermatozoa. It has properties similar to the DPNase obtained from potatoes by Kornberg and Pricer *(10)*. These DPNase enzymes split the pyro-phosphate linkage of DPN, TPN, ATP, and FAD and do not require Ca^{++} for activation. Neither are they inhibited by nicotinamide, as is the DPNase from brain tissue, which catalyzes the rupture of the nicotinamide-ribose linkage, according to Handler and Klein *(5)* and Swingle *et al. (21)*.

Results of the experiments with bull spermatozoa, in which Ca^{++} and nicotinamide were added to "spermatozoan succinic dehydrogenase-estimating" mixtures in this investigation, indicate the possible existence in spermatozoa of a DPNase similar to that of brain tissue, and its removal by washing of the spermatozoa (Table 3). It is also evident that nicotinamide significantly decreases succinic dehydrogenase activity in unwashed spermatozoa but does not do so in washed cells in the presence of added DPN. Similarly, Ca^{++} ions appear to be slightly inhibitory when added to washed spermatozoa, and slightly stimulatory toward unwashed spermatozoa (Table 3).

DISCUSSION

Results of this investigation demonstrate that the washing of bull spermatozoa with isotonic saline solution results in a highly significant stimulation of their succinic dehydrogenase activity. Additional data obtained by Smith *et al. (20)* indicate that this effect may be subject to a seasonal variation. Koefoed-Johnson and Mann *(9)* have observed an increase in O_2 consumption by spermatozoa, with membranes damaged by detergents in the presence of succinate as a subtrate. Their data would indicate that this increase in succinic dehydrogenase activity might be the result of the membrane damage. However, the data obtained in this investigation suggest other interpretations. Results reported herein and by Smith *et al. (19, 20)* indicate the existence of a seasonal fluc-

tuation in DPN, and possibly in cholesterol and carotene, levels in bull semen. Superimposed is the effect produced by the removal of DPN, DPNase, and the upset in the balance of enzymes when spermatozoa are washed or excessively diluted. A combination of these factors may be the cause of the observed stimulation and inhibition of succinic dehydrogenase activity of bull spermatozoa.

The harmful effect of washing appears to be the result of an upset in the delicate balance of coenzymes, enzymes, and activators which may be washed from the spermatozoa during the procedure. The dilution effect, then, may be the result of the removal from the spermatozoa and the excessive dilution of the coenzymes, enzymes, and activators, which diminishes their effectiveness. The increased succinic and malic dehydrogenase activity following washing may be owing, then, to the removal of DPN and/or DPNase. These postulates are based upon (a) the observed significant inhibition of succinic dehydrogenase activity of unwashed spermatozoa, but not of washed spermatozoa, by the addition of nicotinamide or DPN; (b) the slight stimulation of the succinic dehydrogenase activity of unwashed spermatozoa in contrast to the slight inhibition of the activity of this dehydrogenase by the addition of Ca^{++}, and (c) the demonstrated inhibition of succinic dehydrogenase activity of washed spermatozoa by the addition of DPN.

Removal of inhibition of spermatozoan succinic dehydrogenase by DPN upon the addition of *l*-glutamate, strengthens the evidence that DPN is responsible for the inhibition of spermatozoan succinic dehydrogenase activity. Larson and Salisbury *(11)* have reported that seminal plasma contains a higher concentration of glutamic acid than any of the other amino acids. The high level of glutamate may be an automatic regulatory mechanism for spermatozoa, allowing an active succinic dehydrogenase to function in undiluted semen, even in the presence of optimum DPN concentrations. Therefore, the incorporation of glutamate in a semen diluent is suggested.

It was necessary to compare the aldolase levels in washed and unwashed spermatozoa, since prior removal of aldolase by washing would alter the validity of the dehydrogenase determination. Results obtained show that the aldolase activity of spermatozoa is not lowered by washing of the spermatozoa. Hence, the substrate cited could be used for the dehydrogenase determinations.

If, as some investigators assume, the energy of motility for spermatozoa is obtained predominantly from glycolytic metabolism, then the demonstration of a highly significant reduction in the glyceraldehyde-3-phosphate dehydrogenase activity by washing would indicate that this enzyme might be of importance in considering the effect of dilution on spermatozoan metabolism, assuming similar effects are produced by dilution and by washing. It may be recalled that White *(22-25)* explained the function of K^{++} in the removal of glycolytic inhibition resulting from washing, by assuming that it had an activating effect upon transphosphorylase, the second phosphate-esterifying enzyme of the glycolytic cycle. But the total reaction catalyzed by the glyceraldehyde-3-phosphate dehydrogenase is the first of these phosphate-esterifying reactions; therefore, demonstration of the removal of this enzyme by washing appears to be of considerable

importance. If, as it has been assumed, washing and dilution are similar in their effect upon spermatozoan metabolism, then the addition of an exogenous source of glyceraldehyde-3-phosphate dehydrogenase to a spermatozoan diluent would appear to be indicated.

SUMMARY

Effect of the washing of bovine spermatozoa four times with isotonic saline solution was determined by a manometric technique.

A highly significant stimulation of succinic dehydrogenase activity of bovine spermatozoa was observed, and its possible relationship to the removal of DPN by the washing treatment has been discussed. Addition of *l*-glutamate to semen diluents under certain conditions is indicated.

An apparent slight, though not statistically significant, stimulation of malic dehydrogenase activity of bovine spermatozoa was observed, which seemed to be related to the removal of DPNase by washing of the spermatozoa.

A highly significant inhibition of glyceraldehyde-3-phosphate dehydrogenase activity was obtained, which appeared to be the result of removal of this soluble enzyme by washing of the spermatozoa. Addition of an exogenous source of glyceraldehyde-3-phosphate dehydrogenase to semen diluents was suggested. The suggestions made on the basis of the results obtained in this investigation are being subjected to test.

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DENSITY AND SIZE AS FACTORS AFFECTING PASSAGE RATE OF INGESTA IN THE BOVINE AND HUMAN DIGESTIVE TRACTS[1]

K. W. KING AND W. E. C. MOORE
Department of Biochemistry and Nutrition, Virginia Agricultural Experiment Station, Blacksburg

Effect of density and size of particles on their rate of passage through the digestive tract of cattle and humans was estimated with inert plastic particles. The particles that passed most rapidly had a density of about 1.2 gm./cm.^{3} and a size of 20 to 30 $\times$ 10^{-3} cm. *Editor.*

The effect of the physical characteristics of feeds on their rates of passage through the digestive tract has not been evaluated directly. Blaxter *et al. (2)* using sheep, and Balch *et al. (1)* using mature cattle, estimated the effect of comminution on the passage rate of stained hay, but their results were at variance. It was suggested by Blaxter that part of the difference arose from the lower total feed intake in the animals used by Balch. Blaxter's data demonstrate that both increased comminution and increased total intake accelerated passage rate. The same relationship between total intake and excretion rate was observed in goats by Castle *(3)*.

Other investigations of passage rate cited by Castle *(4)* have employed stained oats, straw, hay, and fecal fibers. Moore and Winter *(7)* used ferric oxide and rubber as indicators of passage rate, and Hoelzel *(6)* used rubber, cotton, seeds, glass beads, and several metals. Blaxter *et al. (2)* concluded that the physical characteristics of a feed determine to a large extent its passage rate and thus its digestibility.

This report attempts to evaluate particle size and density as influencing passage rate. Inert plastic materials have been employed to eliminate confounding of the data by progressive diminution in particle size through the gut, alteration in density as a result of hydration or bubble occlusion, and alteration in particle composition through progressive removal of digestible components. Observations on the passage rate of chromic oxide have been included in these studies, because this indicator, like the plastics, but unlike natural feeds, would presumably traverse the digestive tract at constant particle size and density. The inclusion of Cr_2O_3 in these studies was needed for further information about the movement of this indicator, in relation to its use in digestibility trials *(5)*.

EXPERIMENTAL PROCEDURES AND RESULTS

Five three-year-old animals maintained on four pounds of grain daily and mixed hay ad libitum were used. Grade Hereford Steers 1, 2, and 3 were fitted with plastic cannulas in permanent fistulas. Grade Hereford Steer 4 and Guern-

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[1] Contributing to Southern Regional Project S-12.

sey Heifer 5 were not fistulated. The five human subjects were normal males ranging in age from 22 to 30 years. Their dietary regimen was not controlled.

The physical characteristics of the plastic particles [2] are summarized (Table 1). In general, the size ranged between particles 1/8-inch and 3/8-inch on a side. The plastic of density 0.920 gm./cm.^{3} was quite soft and frequently appeared to have been broken up by chewing into pieces too small to recover quantitatively. Some breakage of particles of density 1.18 and 1.24 gm./cm.^{3} was also observed.

TABLE 1

Characteristics of plastic particles used in evaluating effect of particle size and density on passage rate

Trials	Density	Volume	Shape [a]	Weight
	(gm./cm.^{3})	*(cm.^{3}/1,000)*	*(relative dimensions)*	*(mg/particle)*
1, 2, 3	0.92	27.1	1 × 1 × 1	24.9
	1.09	36.0	1 × 1 × 2	39.2
	1.14	42.6	1 × 2 × 4	48.5
	1.18	16.3	1 × 2 × 2	19.2
	1.42	11.5	1 × 1 × 1	16.4
4	1.18	13.5	1 × 1 × 1	16.0
	1.18	20.1	1 × 1 × 2	23.8
	1.18	37.7	1 × 1 × 2	44.5
	1.20	23.4	1 × 1 × 1	28.1
	1.20	40.9	1 × 1 × 2	49.1
	1.20	188.0	1 × 1 × 1	226.0
	1.24	23.0	1 × 1 × 1	28.5
	1.24	41.7	1 × 1 × 2	51.6
	1.24	44.9	1 × 1 × 2	55.7

[a] 1 × 1 × 1 = cube of volume shown in last column.

Total fecal collection was carried out during all trials. In the work with steers, time was recorded for each defecation, and particles were recovered from each dung pile by flotation. The recovery procedure was essentially the following: Each dung pile was washed through 1/4-inch mesh screening into a ten-gallon garbage can and thoroughly mixed with several gallons of water by hand. The can was then filled to within two inches of the top with water. Particles of density 0.920-gm./cm.^{3} were then recovered from the floating material, which was picked up by skimming the top few inches with wire gauze. Contents of the can were stirred again several times during removal of the floating particles. Feed particles less dense than the remaining lightest plastic particles were then removed by passing a stream of water rapidly through a weighted hose into the bottom of the can. After 20 to 30 minutes of this flotation treatment, almost all of the fecal material except the plastic particles and large pieces of grain and heavy hay stems had been removed. The water was decanted off after settling for a few minutes. A second flotation of the residue in a one-liter graduated cylinder allowed easy quantitative recovery of the plastic particles from the fecal residues.

Particles were separated into size and density classes in white light, where there were conspicuous color or shape qualities, or in ultraviolet light, where

[2] Samples of plastic were generously provided by Polychemicals Department, E. I. du Pont de Nemours and Company, Wilmington, Delaware, through Dr. Wayland W. Rennie.

no differences other than the color of the fluorescence were reliable. Each of the density classes could be separated from all of the others by one of these methods.

Recovery of the particles from human feces was accomplished by squeezing small chunks of excreta through 6 ×6-inch wire laboratory gauzes under a gentle stream of cold tap-water. "R-values" were calculated according to Castle *(3)*.

Trials 1, 2, and 3 were designed to evaluate the effect of particle density on passage rate through, respectively, the entire digestive tract of the bovine, the post-ruminal digestive tract of the bovine, and the digestive tract of the human.

In Trial 1, 100 particles of each density (see Table 1) were fed with the grain to the three fistulated animals just before the evening feeding. Total fecal collection was begun at 8 A.M. next morning and continued through a total of 117 hours. At that time the reticulo-rumen of the animal with the lowest fecal recovery of particles (Animal 3) was completely emptied par fistulum. Separation of the top and bottom ingesta was attempted by first removing the hay-mat portion by hand. Bottom ingesta was then recovered by means of a one-inch hose attached to a vacuum line. The organ was flushed several times with water, to insure complete recovery of the particles. The animal's rumen was then reinoculated with half of the fill from each of the other two animals. Recovery procedure for rumen ingesta was essentially the same as that used for fecal samples.

Excretion patterns from Animal 2 are shown (Figure 1). The excretion rate here was intermediate between that of Animals 1 and 3. Top ingesta from the reticulo-rumen of Animal 3 after 117 hours contained 0, 10, 10, 1, and 2 particles of specific gravities 0.92, 1.09, 1.14, 1.18, and 1.42, respectively; the bottom ingesta contained 0, 4, 14, 1, and 35 of the particles, again in order of increasing density. Some residual top ingesta was undoubtedly present in the bottom sample, but the reverse was not true. Summary data from all three animals are plotted (Figure 2B). In this figure the data on specific gravities of 1.20 and 1.24 are from Trial 4, as described below.

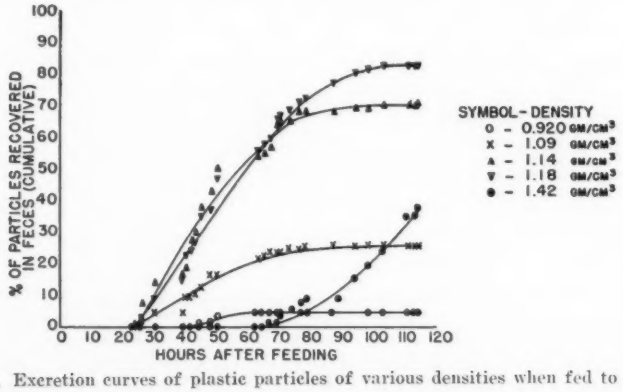

FIG. 1. Excretion curves of plastic particles of various densities when fed to a steer.

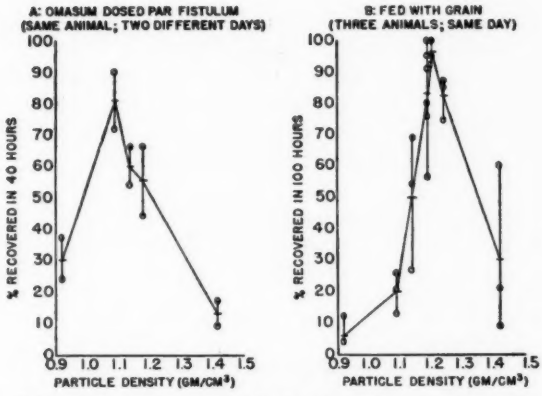
A: OMASUM DOSED PAR FISTULUM
(SAME ANIMAL; TWO DIFFERENT DAYS)
% RECOVERED IN 40 HOURS
PARTICLE DENSITY (GM/CM³)
B: FED WITH GRAIN
(THREE ANIMALS; SAME DAY)
% RECOVERED IN 100 HOURS
PARTICLE DENSITY (GM/CM³)

FIG. 2. Relationship between particle density and excretion rate in cattle.

Trial 2 (run twice at one-week intervals) consisted of placing a gelatin capsule containing 100 particles of each density into the omasum of Animal 2 and recovering the particles from the feces excreted during the next 60 hours. Results of these trials are shown (Figure 2A).

In Trial 3, 25 particles of each density were swallowed in a water slurry by the human subjects. All fecal samples obtained after dosing were checked for plastic particles. Summary data describing the particles present in the first positive fecal sample are given (Table 2). In the human subjects, total recoveries of 83 to 97% were obtained in the first three positive samples. No accurate record of time-of-dosing or time-of-defecation was kept with the human subjects, because toilet habits normally dominate other factors in determining the frequency of human defecation. First appearance of particles ranged between 18 and 72 hours.

Trial 4 examined the passage rate of several sizes of plastic particles of three densities near the optimum, with respect to passage rate as found in Trials 1, 2, and 3. The data (Table 1) describe the physical characteristics of the particles used.

Each of three animals (2, 4, and 5) was fed 100 particles of each size and density mixed with grain at the evening feeding. Beginning at 8 A.M. the next

TABLE 2

Recovery of plastic particles of several densities from human subjects in the first positive defecation after feeding [a]

Density	Subject					
	1	2	3	4	5	Av.
0.92	6	19	5	6	7	8.6
1.09	13	25	20	16	16	18.0
1.14	12	19	15	9	11	13.2
1.18	20	23	12	11	9	15.0
1.42	14	20	12	7	4	11.4

[a] Twenty-five particles of each density were fed.

morning and for the next 79 hours total fecal collection was made. Particles were recovered by the usual procedure. Plastics of density 1.18 and 1.20 gm./cm.^{3} were broken up appreciably when chewed. As a result, many small particles of these densities were recovered in the feces. These small particles were counted as a separate size group of estimated volume 3×10^{-3} cm.^{3} The data are presented in terms of R-values (Figure 3).

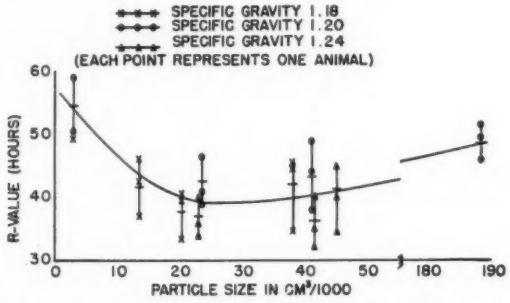

FIG. 3. Passage rate (R-value) of plastic particles through the bovine digestive tract as a function of particle size.

Because of the close relationship of particle passage rates to the practical problems of using indicators, in estimating the nutritive value of livestock feeds, similar analysis of the excretion pattern of chromic oxide from the bovine was made.

Animal 1 was dosed with 15 gm. of Cr_2O_3 in a gelatin capsule placed at the esophageal orifice of the reticulo-rumen. At the same time, and at three-hour intervals for the next 96 hours, one-pint samples of ingesta were taken from the middle of the rumen and refrigerated. These were later analyzed for Cr_2O_3 *(8)*. At 96 hours, a gelatin capsule containing 2.5 gm. of Cr_2O_3 was placed in the omasum. One more sample of rumen ingesta was taken at the 99th hour, to be certain that the second dose of indicator did not move back into the rumen. Total fecal output at natural intervals was measured, and aliquots of each defecation were refrigerated for later analysis. Data are shown (Figure 4). Similar results were obtained when the experiment was repeated using Animal 2. R-values for Cr_2O_3 when fed in these two experiments were 33.6 and 35.7 hours, respectively. In contrast, R-values for fecal excretion of Cr_2O_3 placed in the omasum were 19.2 and 18.7, respectively, in these same runs. By curve-fitting, the equations describing the disappearance of indicator from the rumen and its appearance in the feces as functions of time-since-dosing were determined to be of the form $y = ab^x$, where y is the indicator concentration and x is the time since dosing. (These curves are included in Figure 4.) When the experimental curves of the Cr_2O_3 disappearance from either the rumen or the feces were smooth enough, this exponential function was found to fit the data quite closely. The magnitude of constant a is determined largely by the amount

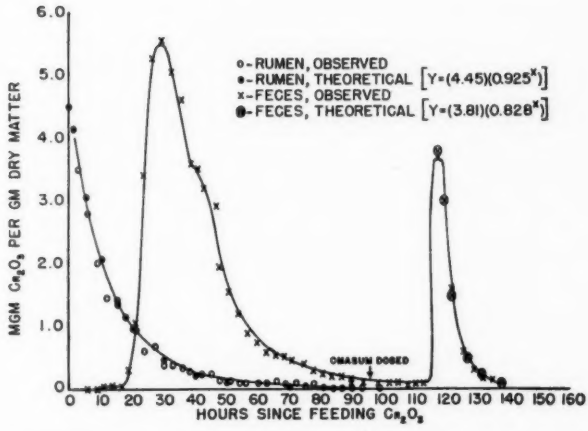

FIG. 4. Disappearance of Cr_2O_3 from the rumen and feces of a steer after feeding the indicator, or placing it directly into the omasum.

of indicator fed. The constant b is invariably less than 1.0, becoming increasingly small as the excretion rate increases. Its magnitude is a reflection of passage rate.

The conspicuous shoulder on the descending portion of the fecal indicator excretion curve in Figure 4 was also present in the data from Animal 2. It appears to result from nonuniform intraruminal distribution of the indicator during the first four to six hours after feeding. The magnitude of this heterogeneity in Cr_2O_3 distribution was demonstrated by placing a gelatin capsule containing 15 gm. of Cr_2O_3 near the esophageal orifice in the reticulo-rumen, by reaching through the fistula. The capsule was placed during the morning feeding of Animal 3. At hourly intervals for the next 13 hours, one-pint samples of rumen ingesta were taken manually from the top, middle, and bottom of the organ, about two feet anterior to the fistula. These samples were then analyzed for Cr_2O_3, with the results shown (Figure 5).

DISCUSSION

The slower passage rate of particles either much lighter or much heavier than specific gravity 1.2 is evident in the digestive tract of the human, more obvious in the longer digestive tract including a large cecum (omasum-dosed steers), and still more conspicuous in the entire ruminant digestive tract. These data indicate that the factors which effect separation in the monogastric tract of the human are probably the same as those which operate more dramatically in the ruminant, where larger organs, more divergent physical pathways of feed, greater volumes of ingesta, and coarser ingesta are found. The simplest explanation of the separation in the human appears to lie in retarding of particles less dense than chyme, in passing through intestinal loops which lie

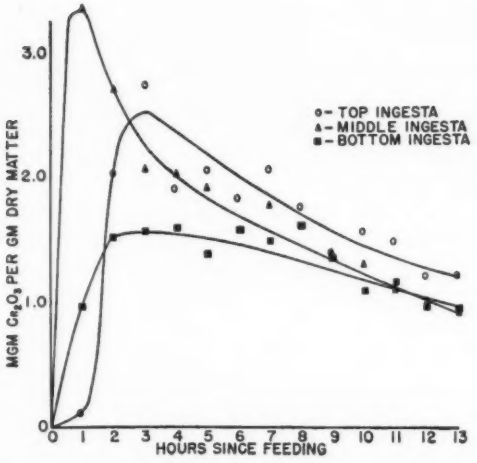

FIG. 5. Intramural distribution of Cr_2O_3 after feeding.

in the position of an inverted "U," while heavy particles tend to pass slowly through intestinal loops lying in the "U" position. In the ruminant, three anatomical differences from the human would tend to accentuate this effect: the longer intestinal tract, the sizable cecum, and the compound stomach. That a great deal of separation occurs in the rumen alone is directly evidenced by the relative numbers of particles of the several densities recovered from the rumen at the end of Trial 1. Here the heavy particles (specific gravity 1.42) were found only in bottom ingesta; almost all of the particles of specific gravity 1.09 had been regurgitated and chewed for cud.

A consistent observation throughout these studies was that none of the particles was ever evident on the surface of the feces as excreted. Even residual hay particles were seldom seen. Apparently some mechanism exists whereby these large particles are packed into the body of the fecal mass, thereby minimizing irritation of the lower intestinal mucosa.

The complete significance of the close fit between the Cr_2O_3 excretion curves and equations of the family $y = ab^x$ is not certain. The magnitude of the constant a, although largely dependent upon the amount of indicator administered, must also reflect such factors as total feed consumption, digestive efficiency, and degree of mixing of the indicator with ingesta in the reticulorumen and cecum. The magnitude of term b, which is actually an estimate of both the passage rate and the compactness of the indicator-laden portion of ingesta, should also be influenced by digestive efficiency, intestinal motility, and total feed intake, but not by the amount of indicator.

The elevated concentration of Cr_2O_3 in top ingesta (Figure 5) may represent either adsorption of the indicator onto roughage particles, or lodging of indicator particles in pits and crevices of the fiber. Whether either or both of

these processes occur has not been established, but Cr_2O_3 does appear to move with the fibrous rather than the soluble constituents of feed. Considering only that Cr_2O_3 has a specific gravity of 5.1 and is very finely dispersed, the indicator would be expected to move very slowly through the digestive tract on the basis of the movement of plastic particles (summarized Figure 3). This not being true (R-values of approximately 34 hours), factors other than density and particle size must be dominant in determining passage rate of the indicator. Perhaps adsorption or occlusion of Cr_2O_3 on hay particles of more optimum density and size is the deciding factor.

A probable basis can be offered for the shoulder on the excretion curve when Cr_2O_3 is fed (Figure 4). The shoulder appears to be the result of rumen function, because it is not evident in the excretion pattern when the indicator is placed in the omasum. Figure 5 demonstrates that the indicator reaches quite high transitory concentrations in the rumen ingesta, near the omasal orifice, just after feeding ("middle ingesta" curve of Figure 4). During this two- or three-hour period, ingesta richer in indicator than most of the rumen contents passes on to the omasum. Eventually this indicator appears in the feces, in the sharply rising and falling excretion pattern typical of omasum-dosing, as seen during the 96th through 140th hours (Figure 4). It is this behavior to which the portion of the fecal curve prior to the shoulder is attributed when the indicator is fed. The indicator which has distributed throughout the reticulo-rumen is excreted in a more gently rising-and-falling pattern. On the basis of this hypothesis, the fecal excretion curve reflects the sum of these two curves, and the shoulder results from the lag in time between the high concentrations of indicator entering the omasum just after feeding, and excretion of the rest of the indicator after mixing with rumen ingesta.

SUMMARY

Effect of density and size of the passage rate of particles through the digestive tract of cattle and humans has been estimated, using inert plastic particles. An optimum (maximum passage rate) in density at approximately 1.2 gm./cm.^{3} and in size between 20 and 30 $\times$ 10^{-3} cm.^{3} has been observed.

The excretion pattern of chromic oxide by cattle has been found to follow the equation $y = ab^x$, in general, but the rate of passage is faster than would be predicted from its size and density. The significance of this equation and of a pronounced shoulder in the excretion curve has been discussed.

ACKNOWLEDGMENTS

The possibility of using plastic particles of known density and size to evaluate the effect of these characteristics on passage rate in the bovine arose during a conversation between the authors and Drs. R. W. Engel, R. E. Blaser, and W. A. Hardison of this experiment station. P. H. Smith, J. H. Hill, and J. R. Bartlett assisted greatly in sampling the animals and processing fecal matter. Chromic oxide analyses were made by the late W. N. Linkous. The authors deeply appreciate the generosity of these people.

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FERTILITY RESULTS WITH FROZEN SEMEN STORED UP TO ONE YEAR[1]

J. P. MIXNER AND S. H. WIGGIN[2]

New Jersey Agricultural Experiment Station and
Sussex County Cooperative Breeding Association, Inc., Sussex

Semen frozen at –79° C. in a diluter of egg yolk, sodium citrate, glycerol, streptomycin, and water had a mean fertility after 7-14 days of 66.7%, after 6 months of 70.1%, and after 12 months of 65.7%. The fresh semen had a fertility of 68%. *Editor.*

There are few data indicating in what manner fertility of bull semen frozen and stored at about −79° C. is influenced by length of storage period. Rowson and Polge *(6)* found that a storage period of from one to 12 months at −79° C. and stored at about –79° C. is influenced by length of storage period. Rowson *et al.* *(1)* found that the fertility of three bulls' semen, frozen and stored at −79° C. for 103 days, showed no decline when compared to frozen semen stored at −79° C. for one day only. Snyder *et al.* *(7)* reported that the fertility rate of 11 bulls' frozen semen stored for from ten to 140 days was 67%, expressed as 60- to 90-day nonreturns to first service; whereas, part of the same semen stored for another 122 days had a nonreturn rate of 58%.

Bruce *(2)* reported that the fertility of six semen samples from each of two Shorthorn bulls showed no decline after freezing and storage for two years. Hendrikse *et al.* *(3)* found no evidence of a decline in fertility of frozen semen from 22 bulls tested at intervals of one month for five months and from five of these bulls tested at monthly intervals up to 12 months. Madden *(4)* obtained a fertility rate of 60.5% on 17,433 breedings to frozen semen stored less than two months, and a fertility rate of 58.0% on 13,352 breedings to frozen semen stored over two months—a highly significant difference statistically.

This paper is a preliminary report on a projected long-time study of the effects of length of storage at −79° C. on fertility of dairy bull semen.

MATERIALS AND METHODS

Semen ejaculates were collected from five Holstein sires in the stud at the Dairy Research Farm, New Jersey Agricultural Experiment Station, Sussex, which is the source of Holstein and Guernsey semen used by the Sussex County

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[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University, the State University of New Jersey, Department of Dairy Industry, New Brunswick.

[2] Manager, Sussex County Cooperative Breeding Association, Inc., Sussex, New Jersey.

Cooperative Breeding Association, Inc. The ejaculates were split, part for use as unfrozen control semen and part for use as experimental frozen semen. The diluter for the unfrozen control semen had a final concentration of 20% egg yolk, 2.175% sodium citrate dihydrate, and 0.5-mg. of streptomycin sulfate per ml. The frozen semen was processed, frozen, and stored at —79° C. by the methods and equipment outlined by Mixner *(5)*. The diluter for the frozen semen had a final concentration of 20% egg yolk, 2.175% sodium citrate dihydrate, 7% glycerol, and 0.5-mg. of streptomycin sulfate per ml. Samples for both control and frozen semen were diluted to obtain a minimum of 15 million motile sperm per ml. (fresh and immediately post-freezing).

The unfrozen control semen was inseminated on the day of collection and on the day following; a portion of the experimental frozen semen was inseminated after storage periods of from seven to 14 days, six months, and 12 months by technicians of the Sussex County Cooperative Breeding Association, Inc. The remaining frozen semen will be used for future fertility trials, the next trials being scheduled subsequent to two years of storage. Ampoules of frozen semen were packed in dry ice, in pint-sized vacuum jars, for transportation from storage to the farms where the semen was to be used for breeding. Immediately before use the ampoules were melted in cold or cool water (5 to 15° C.).

The per cent of first-service cows which were not returned for service 30 to 60 days and 60 to 90 days after service, as determined from routine association records, were used as measures of fertility.

RESULTS

Various characteristics of the semen samples initially and after dilution and freezing are shown (Table 1). In all instances there was an estimated minimum of 13 million motile sperm per ml. of diluted semen, as determined immediately after freezing.

Table 2 presents the fertility results on these trials, nonreturns to service being tabulated both at 30 to 60 days and 60 to 90 days after breeding. The

TABLE 1

Characteristics of five bulls' semen initially and after freezing

	Semen ejaculates			Motile sperm			
				Diluted fresh semen[a]		Frozen semen[b]	
Bull No.	No.	Total volume	Initial sperm concentration ($\times 10^6$)		Estimated number ($\times 10^6$)		Estimated number ($\times 10^6$)
		(ml.)	*(No/ml)*	*(%)*	*(No/ml)*	*(%)*	*(No/ml)*
24H	1 + 2	15.7	1213	65	15	45	22
H101	1 + 2	6.2	1783	60	14	40	13
H88	2	8.1	1325	70	15	45	15
H89	1 + 2	8.0	1600	60	19	40	16
H103	2 + 3	15.2	674	60	15	50	25
Means		10.6	1319	63	15.6	44	18.2

[a] Immediately after dilution.
[b] Immediately after freezing.

TABLE 2

Effect of length of storage of frozen semen on its fertility

Bull and semen sample	Period after breeding	Unfrozen control semen		Frozen experimental semen stored for 7 to 14 days		Frozen experimental semen stored for 6 months		Frozen experimental semen stored for 12 months	
		Cows bred	N.R.[a]	Cows bred	N.R.[a]	Cows bred	N.R.[a]	Cows bred	N.R.[a]
	(days)	*(No.)*	*(%)*	*(No.)*	*(%)*	*(No.)*	*(%)*	*(No.)*	*(%)*
24H–51	30–60	38	84.2	20	70.0	19	84.2	22	54.5
	60–90		78.9		65.0		78.9		54.5
H101–54	30–60	39	69.2	21	85.7	22	86.3	20	90.0
	60–90		64.1		80.9		77.3		90.0
H88–60	30–60	42	81.0	19	84.2	17	70.6	19	73.7
	60–90		78.5		78.9		70.6		73.7
H89–68	30–60	32	50.0	20	50.0	21	71.4	21	57.1
	60–90		50.0		40.0		66.7		57.1
H103–69	30–60	24	66.7	19	68.4	18	55.6	20	65.0
	60–90		62.5		63.1		55.6		55.0
Totals	30–60	175	71.4	99	70.7	97	74.2	102	67.6
	60–90		68.0		66.7		70.1		65.7

[a] N.R.—first-service cows not returned to service.

level of fertility of the frozen semen at all storage-time intervals approximated that of the fresh semen, there being no differences statistically between the various mean nonreturn rates. The mean declines in the per cent of nonreturns to first service between 30 to 60 days and 60 to 90 days after service varied from 1.9 to 3.4 in the various groups, and were considered to be of normal magnitudes.

Although the data are limited in amount, they do indicate satisfactory fertility results with frozen semen stored for as long as one year, through the use of methods and procedures now employed generally.

SUMMARY

Five split ejaculates of semen from five Holstein bulls were used to determine the effects of freezing and the length of storage of frozen semen at —79° C. on the fertility of the semen. The semen was frozen in a diluter composed of egg yolk, sodium citrate, glycerol, streptomycin, and water. The mean fertility of the fresh control semen (175 cows) was 68.0%. For the frozen semen after storage at —79° C. for 7 to 14 days (99 cows), 6 months (97 cows), and 12 months (102 cows), the mean fertility was 66.7%, 70.1%, and 65.7%, respectively, as measured by the number of first-service cows not returned for service 60 to 90 days after insemination.

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VARIATION IN PLASMA PROTEIN-BOUND IODINE LEVELS IN DAIRY CATTLE DUE TO ANIMAL, DAILY, DIURNAL, AND METHOD EFFECTS[1]

H. D. LENNON, JR., AND J. P. MIXNER
New Jersey Agricultural Experiment Station, Sussex, N. J.

The effects of the animal, daily and diurnal rhythm, and assay errors on the plasma PBI levels were studied. The main variations were due to the animal, blood sampling, and methodology, amounting to 87.8, 9.7, and 2.5%, respectively, of the total variance. One blood sample per animal is sufficient in routine plasma PBI investigations. *Editor.*

The level of plasma protein-bound iodine (PBI) has become an important index of thyroid function in the human *(8, 10, 12)*, but its usefulness in dairy cattle has not become established. One reason for this is the lack of a recognized, well-defined syndrome involving the thyroid. Also, it is not feasible to compare routinely the level of plasma PBI with any other established measure of thyroid activity, such as basal metabolic rate. Therefore, an evaluation of the usefulness of plasma PBI levels in dairy cattle (as a measure of thyroid activity) must be based primarily on its relation to the physiological status of the animal, particularly on its relation to productive and reproductive characteristics.

Several investigators have reported on the normal ranges of plasma PBI values for various age groups of cattle *(5, 6, 7)*, breed differences *(5, 7, 9)*, seasonal trends *(1, 5)*, and the relationship between plasma PBI level and certain measures of growth *(3)*. Only one preliminary study by Lennon and Mixner *(4)*, however, has dealt with some of the sources of variation in the determination of plasma PBI in cattle. In this study, the plasma samples were stored at –20°, 2°, and 25° C. for zero, one, three, and six days prior to assay. The variance associated with the storage of plasma constituted 25% of the total; whereas, method error variance contributed only ten per cent to the total variance.

In order to study the relationship between plasma PBI levels and physiological traits in dairy cattle, it becomes important to evaluate short-time variation in levels of protein-bound iodine as a basis for indicating the time and frequency of blood sampling. Accordingly, the object of this study was to

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determine the relative amounts of variation in plasma PBI levels due to animal effects, possible daily and diurnal rhythm effects, and assay errors.

EXPERIMENTAL PROCEDURE

Four groups of animals were utilized in this study: three pregnant, nonlactating cows; three lactating, nonpregnant cows; three open heifers, 15 to 18 months old; and three heifer calves, 3 to 6 months old. Four animals (one animal from each group) were bled for a week's sampling period, constituting a run. Three runs were conducted during the late fall and winter of 1955–1956.

Blood samples were collected from each animal at 8 A. M., 4 P. M., and 12 P. M. on each of four alternate days, for a total of 12 blood samples per animal. Blood was collected from the mammary vein of the mature animals and from the jugular vein of younger animals. One ml. of anti-coagulant (24 g. ammonium oxalate, 16 g. potassium oxalate, and 1 liter of water) was evaporated to dryness in each 15-ml. Pyrex blood-collection tube. Blood samples were centrifuged immediately after collection to recover the plasma.

The method of Brown *et al. (2)*, with slight modifications *(4)*, was used for plasma PBI determinations. Three separate fusions were performed on each plasma sample and one photometric determination was performed on each fusion tube.

The analysis of variance presented in this study was according to the outline of Snedecor *(13)*. The expectations of mean squares for fixed and random effects were determined by the method of Schultz *(11)*.

RESULTS AND DISCUSSION

Mean plasma PBI values, classified as to group of animals and hour of day at which blood samples were collected, are presented (Table 1). An analysis of variance of the data is given (Table 2). Components of mean squares and estimated variances were determined for the various sources of variation (Table 3). The main sources of variation in this study were grouped under animal effects, blood sampling effects, and effects due to assay methodology (Table 4).

Animal effects in this study are mainly the sum of group, run, and run times group interaction, constituting 23.7, 9.3, and 54.7% of the total variance, re-

TABLE 1

Effect of time of day and physiological status of animals on plasma levels of PBI

Group	Mean plasma PBI values			
	8 A. M.	4 P. M.	12 P. M.	Means
	(μg. %)			
Pregnant, nonlactating cows	3.64	3.89	3.80	3.78
Lactating, nonpregnant cows	4.89	4.98	5.09	4.96
Open heifers, 15 to 18 months old	8.13	8.52	8.06	8.24
Heifer calves, 3 to 6 months old	6.35	6.42	6.49	6.42
Means	5.73	5.95	5.86	5.85

TABLE 2

Analysis of variance of plasma PBI data

Sources of variation	(Symbol)	Degrees of freedom	Mean square	Error term	F
Total		431			
Group	G	3	400.498	GR	2.28
Hour	H	2	1.882	HR	0.78
G × H	GH	6	0.645	GHR	0.92
Run	R	2	119.486	D(R)	56.16[a]
G × R	GR	6	175.871	GD(R)	55.92[a]
H × R	HR	4	2.413	HD(R)	1.01
G × H × R	GHR	12	0.699	GHD(R)	0.56
Day	D(R)	9	2.128	F[GHD(R)]	9.71[a]
G × D(R)	GD(R)	27	3.145	F[GHD(R)]	14.35[a]
H × D(R)	HD(R)	18	2.395	F[GHD(R)]	10.92[a]
G × H × D(R)	GHD(R)	54	1.251	F[GHD(R)]	5.71[a]
Fusions in G × H × D(R)	F[GHD(R)]	288	0.219		

[a] Statistically significant at the 1% level ($P < 0.01$).

spectively (Table 4). Thus, approximately 88% of the total variance may be attributed to variations in plasma PBI values among animals. The total animal variance of 7.69 from this study compares to an animal variance of 1.36 determined in a previous study *(4)*, in which four animals of similar ages and physiological states were used.

The sum of hour, day (within run), and the interactions of hour and day effects with each other and with group and run effects, constitute blood sam-

TABLE 3

Components of mean squares and estimated variances

Source of variance (Symbol)	Expectations of mean squares	Estimated variances (σ^2 or Θ^2)	(%)
G	$\sigma^2F[GHD(R)] + 9\ \sigma^2GD(R) + 36\ \sigma^2GR + 108\ \Theta^2G$	2.08	23.7
H	$\sigma^2F[GHD(R)] + 12\ \sigma^2HD(R) + 48\ \sigma^2HR + 144\ \Theta^2H$	<-0.01	<-0.1
GH	$\sigma^2G[GHD(R)] + 3\ \sigma^2GHD(R) + 12\ \sigma^2GHR + 36\ \Theta^2GH$	<-0.01	<-0.1
R	$\sigma^2F[GHD(R)] + 36\ \sigma^2D(R) + 144\ \sigma^2R$	0.82	9.3
GR	$\sigma^2F[GHD(R)] + 9\ \sigma^2GD(R) + 36\ \sigma^2GR$	4.80	54.7
HR	$\sigma^2F[GHD(R)] + 12\ \sigma^2HD(R) + 48\ \sigma^2HR$	<0.01	<0.1
GHR	$\sigma^2F[GHD(R)] + 30\ \sigma^2GHD(R) + 12\ \sigma^2GHR$	-0.05	-0.5
D(R)	$\sigma^2F[GHD(R)] + 36\ \sigma^2D(R)$	0.05	0.6
GD(R)	$\sigma^2F[GHD(R)] + 9\ \sigma^2GD(R)$	0.33	3.7
HD(R)	$\sigma^2F[GHD(R)] + 12\ \sigma^2HD(R)$	0.18	2.1
GHD(R)	$\sigma^2F[GHD(R)] + 3\ \sigma^2GHD(R)$	0.34	3.9
F[GHD(R)]	$\sigma^2F[GHD(R)]$	0.22	2.5
Total		8.77	100.0

TABLE 4

An evaluation of the main sources of variation in plasma PBI levels

Combined sources of variance	Estimated variances (σ^2 or Θ^2)	(%)
Animal (composed of G, R, and GR)	7.69	87.8
Blood samples—S [Composed of H, D(R), HD(R), and interactions of H and D(R) with G and R]	0.85	9.7
Fusions—F [GHD(R)]	0.22	2.5
Total	8.76	100.0

pling effects and represent primarily short-time variations in levels of plasma PBI. These combined sources of variation represent 9.7% of the total variance (Table 4), mainly through the interaction effects. Diurnal variation, as measured by hour effects, was not statistically significant and constituted a negligible part of the total variance. Daily variation in plasma PBI values was a highly significant effect (Table 2), yet contributed only 0.60% to the total variance.

The variance due to fusion effects was estimated to be 0.22 and accounts for 2.5% of the total variance in this study. In a previous study *(4)*, the variance due to fusion effects was 0.21.

The standard error of a single plasma PBI determination was derived from error term variances consisting of both blood sampling and fusion effects and was found to be the $\sqrt{0.22 + 0.85}$, or 1.04 μg. %, with a coefficient of variation of 17.7%. Table 5 presents the changes in the standard errors (S.E.) and the coefficients of variation of mean PBI determinations as a function of the number of blood samplings and the number of fusion tubes per blood sample. Standard errors were calculated from the following equation based on the error variances in which s equals the number of blood samples collected per animal and f equals the number of fusion tubes per blood sample:

TABLE 5

Effect of varying the number of blood samples per cow, and number of fusion tubes per sample, on the standard errors and coefficients of variation of the appropriate mean PBI values

No. fusions	No. of blood samples					
	1		2		3	
	S. E.	C. V.	S. E.	C. V.	S. E.	C. V.
	(μg.%)	*(%)*	*(μg.%)*	*(%)*	*(μg.%)*	*(%)*
1	1.04	17.7	0.73	12.6	0.60	10.2
2	0.98	16.8	0.69	11.9	0.57	9.7
3	0.96	16.5	0.68	11.6	0.56	9.5

Based on $\sigma^2 S = 0.85$ and $\sigma^2 F[GHD(R)] = 0.22$.
S.E. = standard error of mean PBI determination.
C.V. = coefficient of variation of a mean PBI determination.

$$\text{S.E.} = \sqrt{\frac{0.85}{s} + \frac{0.22}{s \times f}}$$

It can be seen (Table 5) that the standard error of a mean plasma PBI determination and coefficient of variation can be reduced most effectively by increasing the number of blood samplings; whereas, relatively little is gained by assaying multiple-fusion tubes. However, since animal effects constituted a major part (88%) of the total variance, it is suggested that one blood sample per animal would suffice at any given time in routine plasma PBI investigations.

SUMMARY

A study was made of animal, daily, diurnal, and method sources of variations in plasma protein-bound iodine (PBI) levels in dairy cattle. Four groups of

animals, based on age and physiological state, were sampled at 8 A. M., 4 P. M., and 12 P. M. on each of four alternate days for a week. Three separate fusions were performed on each plasma sample and one photometric determination was made on each fusion tube. The three main sources of variation studied were animal, blood sampling, and method effects, constituting 87.8, 9.7, and 2.5% of the total variance, respectively.

Since no diurnal variation in plasma PBI levels could be demonstrated, blood samples for plasma PBI determinations could be collected at any time during the day.

The standard error and the coefficient of variation of a mean plasma PBI determination, derived from error variances, can be reduced most effectively by an increase in the number of blood samples in a given period of time, in preference to more fusion tubes per blood sample. The desired accuracy in plasma PBI determinations must be carefully weighed against the increased work-load in determining the frequency of blood sampling.

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SOME PROPERTIES OF ALKALINE PHOSPHATASE OF MILK [1]

W. HAAB AND L. M. SMITH[2]

Department of Dairying, University of Alberta, Edmonton, Canada

Optimum conditions for the accurate determination of phosphatase in raw milk were determined. The optimum pH of the phosphatase reaction was higher when disodium phenyl phosphate was increased, but it did not shift when sodium β-glycerophosphate was increased. The hydrolysis of disodium phenyl phosphate required about 9,000 cal/mole. The enzyme was most stable at about pH 6.7. *Editor.*

The alkaline phosphatase of milk is of unique interest in the physiology of milk secretion and pasteurization. Literature reviews *(2, 4, 5, 17)* include many papers on phosphatase tests to detect the efficiency of pasteurization. However, only a few fundamental studies of the kinetic and other properties of milk phosphatase have been reported. Folley and Kay *(7)* studied the alkaline phosphomonoesterase of guinea pig mammary gland and found it to be identical with the phosphatase of the kidney tissue. Morton *(18, 19)* and Zittle and DellaMonica *(26, 27)* compared the properties of purified alkaline phosphatases from cow's milk and calf intestinal mucosa and concluded that the enzymes are distinctly different. Kannan and Basu *(12, 13)* used concentrated phosphatase extracts from the milk of cows, buffaloes, goats, and sheep to study the optimum pH, the effect of activators, and the energy of activation of hydrolysis of disodium phenyl phosphate.

Thermal destruction of the enzyme in milk has received considerable study *(10, 11, 13, 14, 23)*, but little information *(21)* is available on its other properties in the original medium. Haab and Smith *(9)* reported on variations in phosphatase activity of raw milk and their possible importance in pasteurization standards. The present paper deals with the phosphatase activity of raw milk as influenced by various factors known to affect enzymatic catalysis in general. Optimum conditions necessary for the accurate determination of phosphatase activity in raw milk are given.

METHODS

Milks of individual cows were obtained from the University herd, and pooled milks, representative of approximately 500 cows, from a local milk plant.

In some of the kinetic studies, phosphatase activity was determined with two substrates—disodium phenyl phosphate and sodium β-glycerophosphate.

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[2] Present address: University of California, Davis.

The modified method of Sanders and Sager described elsewhere *(9)* was used to establish the optimum experimental conditions for the enzymatic hydrolysis of disodium phenyl phosphate. Where the substrate was sodium β-glycerophosphate, the method employed was similar in principle to Gould and Schwachmann's *(8)* technique for the determination of alkaline phosphatase activity in blood serum.

Phosphatase activity is expressed as micrograms phenol or micrograms phosphorus (phosphate) liberated by the enzyme in 0.5 ml. of milk under the described conditions. Experimental error is estimated to be not more than 2%.

Hydrogen ion concentrations were determined with a Model G Beckman glass electrode meter. The measurements were made at 37.5° C., except where otherwise stated.

RESULTS AND DISCUSSION

Effect of pH and substrate concentration on phosphatase activity. The optimum pH for enzymatic hydrolysis depends on various factors, including the substrate, the concentration of the substrate, and the buffer system. A series of buffers, containing varying proportions of boric acid and barium hydroxide, was prepared to give a range of pH from approximately 8.5 to 10.5 when used in the modified *(9)* Sanders and Sager phosphatase determination for raw milk. The pH values of the reaction mixtures were determined at 0, 10, 20, and 30 minutes during the hydrolysis period and were averaged. Figure 1 shows that the apparent pH optimum of the hydrolysis of disodium phenyl phosphate under these conditions is at pH 10.0. This agrees with the hydrolysis pH specified in the Sanders and Sager *(22)* method. The curves

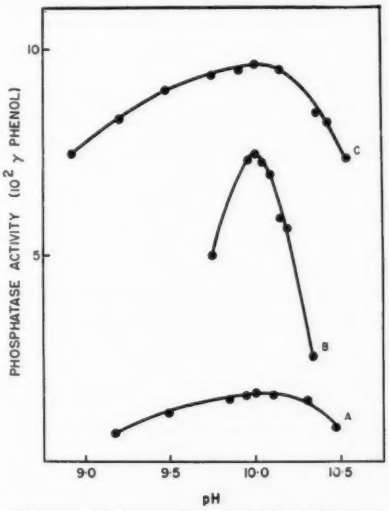

FIG. 1. Apparent pH optimum of the hydrolysis of 0.00834 *M* disodium phenyl phosphate by phosphatase in milk from an individual cow (A), in colostrum (B), and in pooled milk (C).

illustrate the importance of accurate pH adjustment in the determination of phosphatase activity in milk and colostrum.

Hetrick and Tracy *(11)* and Ritter *(20)* found that the 0.00834 *M* concentration of disodium phenyl phosphate in the hydrolysis mixture recommended by Sanders and Sager *(22)* did not saturate the amount of phosphatase in raw milk. To study the effect of substrate concentration on hydrolysis, phosphatase activity was determined at pH 10.0, employing different levels of disodium phenyl phosphate. Figure 2 shows that 0.00834 *M* substrate does not give a zero-order reaction as required for accurate enzyme estimation.

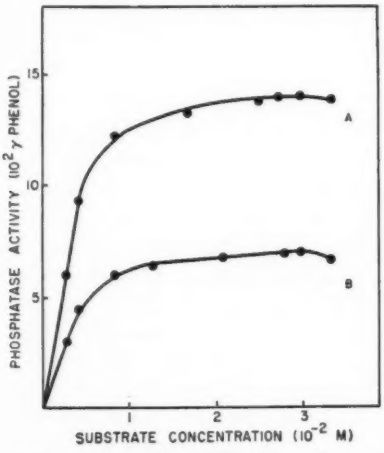

FIG. 2. Effect of increasing concentration of disodium phenyl phosphate on phosphatase activity at pH 10.0 of pooled milk (A) and milk from an individual cow (B).

Because of the possible influence of substrate concentration on the optimum pH of hydrolysis, the latter was redetermined. Table 1 shows the variation in phosphatase activity of an individual and a pooled milk, where the average pH of the catalyzed reaction varied and three disodium phenyl phosphate concentrations were employed. The optimum pH value was 10.32 ± 0.02 for the optimum substrate concentration of 0.02919 *M*. Additional experiments with different milks confirmed these results.

The increase in the pH optimum of the catalyzed reaction with increasing substrate concentration is further illustrated (Figure 3). Maximum hydrolysis for each concentration occurred within a relatively narrow pH zone. Folley and Kay, who worked with alkaline phosphatase obtained from mammary gland tissue of guinea pigs, observed a similar shift of the pH optimum toward the acid side with decreasing substrate concentration. Their postulation that the enzyme may be protected against hydroxyl ions by certain substrates provides an explanation for these results.

When the substrate was sodium β-glycerophosphate, increased substrate concentration did not produce a shift of pH optimum. For each of 11 sub-

TABLE 1

Effect of average hydrolysis pH and concentration of disodium phenyl phosphate on phosphatase activity

Milk	Av. hydrolysis pH	Phosphatase activity (γ phenol)		
		0.02502 *M*	0.02919 *M*	0.03323 *M*
Individual	10.02	223	263	183
	10.10	246	273	201
	10.17	272	288	220
	10.27	289	291	239
	10.33	272	297	261
	10.38	255	288	281
	10.52	——	261	253
Pooled	10.00	1,030	1,290	874
	10.18	1,060	1,330	1,260
	10.27	1,310	1,340	1,280
	10.32	1,240	1,390	1,300
	10.39	1,170	1,350	1,330
	10.47	1,040	1,220	1,300

strate concentrations ranging from 0.004139 to 0.038702 *M* the optimum pH was 9.60 ± 0.02.

Optimum hydrolysis temperature. Phosphatase activity was determined at seven temperatures ranging from 30° to 50° C. The hydrolysis period was 30 minutes and the disodium phenyl phosphate concentrations were 0.00834 *M* and 0.02919 *M*. Figure 4, representative of the results obtained with both individual and pooled milks, shows the data for an individual milk. The optimum hydrolysis temperature was 38.5° C. for both substrate levels, but the curve for the higher concentration exhibits the sharper peak. This decrease in activity on either side of the temperature optimum indicates the importance of keeping hydrolysis temperature between 38° and 39° C.

Length of hydrolysis period. To establish the optimum reaction time, activity determinations were made after hydrolysis of 0.02919 *M* disodium phenyl phosphate at 38.5° C. and pH 10.32 for periods ranging from five to 75 minutes.

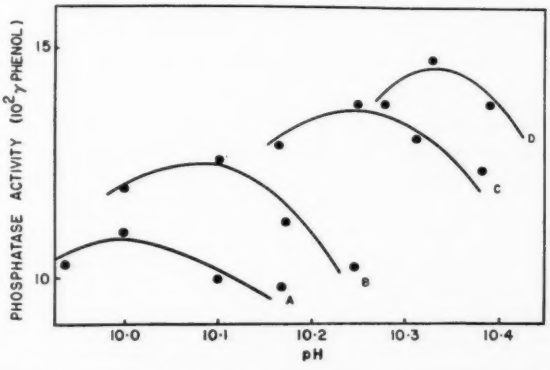

FIG. 3. Shift of optimum hydrolysis pH with different concentrations of disodium phenyl phosphate: 0.00834 *M* — (A), 0.01668 *M* — (B), 0.02502 *M* — (C), 0.02919 *M* — (D).

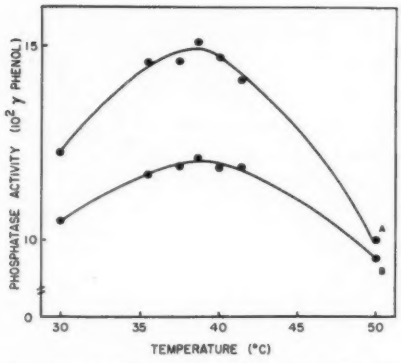

FIG. 4. Effect of temperature on hydrolysis of different concentrations of disodium phenyl phosphate by milk phosphatase: 0.02919 *M* — (A), 0.00834 *M* — (B).

Figure 5 presents data obtained with pooled milk and shows that the hydrolysis period in the phosphatase determination of raw milk should not be longer than 20 minutes, to avoid deviation from zero-order kinetics. Milks from individual cows gave similar results. No attempt was made to investigate possible reasons for the decrease in activity observed for longer reaction times, such as decreased temperature stability of the enzyme or inhibition by reaction products.

The authors recognize that longer hydrolysis times may be desirable to increase the sensitivity of phosphatase tests used in pasteurization control *(22)*, where only trace amounts of residual enzyme are present.

Substrate affinity. An attempt was made to study the affinity between alkaline phosphatase of milk and different substrates. Preliminary experiments established optimum conditions for the hydrolysis of sodium β-glycerophosphate. The Michaelis constant, K_m, was determined by the graphical method of Lineweaver and Burk *(15)*. Figure 6 is the "double reciprocal" plot for disodium phenyl phosphate. K_m was 0.00340 *M* for disodium phenyl phosphate and 0.00560 *M* for sodium β-glycerophosphate.

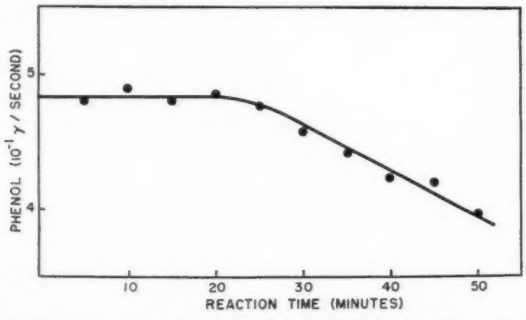

FIG. 5. Effect of reaction time on rate of hydrolysis of disodium phenyl phosphate by milk phosphatase.

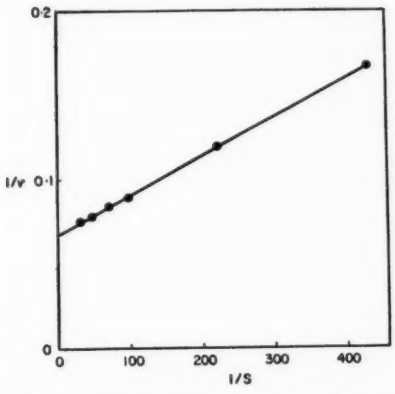

FIG. 6. Effect of substrate concentration on the activity of milk toward disodium phenyl phosphate. S = substrate molarity and v = relative reaction velocity.

According to the Michaelis-Menten *(16)* concept of enzyme-substrate complex, in the so-called "steady state," the rate at which the complex is decomposing is equal to the rate at which it is formed and $1/K_m$ is equal to $k_1/(k_2 + k_3)$. Assuming that k_3 is much smaller than k_2, $1/K_m$ becomes an affinity constant. The above results indicate that the alkaline phosphatase of milk has a greater affinity for disodium phenyl phosphate than for sodium β-glycerophosphate. Delory and King *(6)* explained similar observations for phosphatase from dog feces on the basis that the affinity between the enzyme and substrate increases with decreasing pK of the substrate.

Morton *(19)* found the lower K_m value 6.1×10^{-4} for hydrolysis of disodium phenyl phosphate by purified milk phosphatase. The lack of agreement of the two values may be attributed to possible differences in the state of the enzyme and to different hydrolysis conditions.

Energy of activation. The energy of activation of the hydrolysis of disodium phenyl phosphate was calculated by the empirical equation derived by Arrhenius *(3)*. The specific reaction velocities at 27° and 37° C., K_1 and K_2, respectively, for two individual milks and one pooled milk were determined. The resulting average value of 1.4 for the temperature coefficient Q_{10} is within the normal range for enzyme reactions *(25)*. The average activation energy was 8,970 ± 100 cal / mole. Log K is plotted against the reciprocal of the absolute temperature (Figure 7). The middle point, which is the log K value determined at 32° C., shows that the linearity required by the Arrhenius equation has been met. Kannan and Basu *(13)* reported an activation energy of 8,500 cal / mole for a concentrated phosphatase extract from the milk of Indian cows.

Effect of pH on stability of the enzyme. Preliminary experiments showed that the phosphatase activity of raw milk remained constant for at least 72 hours when the samples were held at 4° C. The relation between hydrogen ion concentration and phosphatase stability was studied by holding a series

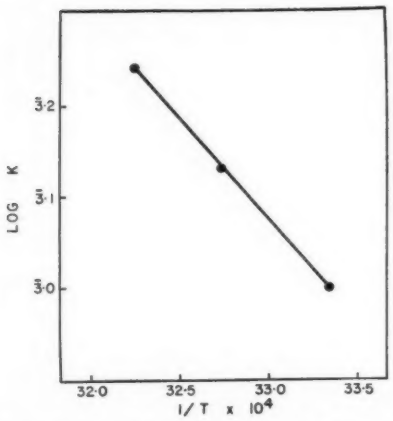

FIG. 7. Plot of log K vs. $1/T$ used in determination of activation energy. K = specific reaction rate, and T = Absolute temperature.

of milk samples, adjusted to different pH values, for one- and two-hour periods at 38° C. Then the phosphatase activity of each sample was determined using optimum conditions, i. e., 0.02919 M disodium phenyl phosphate and hydrolysis pH, temperature and time of pH 10.32, 38.5° C. and 20 minutes, respectively. Figure 8 shows that enzyme activity dropped rapidly on both sides of the normal pH range of milk. Although it would seem preferable to measure phosphatase activity near pH 7.0, where the enzyme is stable, this is not prac-

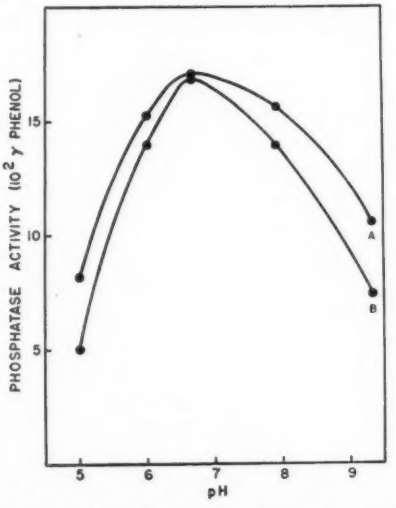

FIG. 8. Effect of pH on the stability of milk phosphatase in the absence of substrate. Milk held at indicated pH values for one hour (A) and two hours (B).

TABLE 2
Partial reactivation of acid-inactivated milk phosphatase

Milk brought to pH 5.2	Phosphatase activity (γ phenol)			
	Original	After inactivation for 2 hours at pH 5.2	After reactivation for 6 hours at	
			pH 6.7	pH 9.5
In 14 hours by souring at 20° C.	1,800	589	820	674
In 5 seconds by addition of 0.1 *N* HCl	1,800	519	627	561

tical because phosphatase has little activity toward substrates at this pH. Folley and Kay *(7)* have made similar observations with guinea pig mammary gland phosphomonoesterase.

To investigate the reactivation of milk phosphatase partly inactivated by acid, two samples of the same pooled milk were adjusted to pH 5.2, one by souring at room temperature and the other by adding 0.1 *N* HCl. Both samples were held at 38° C. for two hours. Then the residual phosphatase activities were determined, and portions of each sample adjusted to pH 6.7 and 9.5 with 0.1 *N* NaOH. After six hours at 38° C., the phosphatase activities were again determined. Table 2 summarizes the data.

Slow souring resulted in slightly less inactivation of the enzyme than did the direct addition of acid. The samples held at pH 6.7 regained more of their lost phosphatase activity than did those held at pH 9.5. These results are in agreement with the observations of Sjöström *(24)*.

Sjöström has reviewed various theories explaining acid inactivation and restoration of phosphatase activity. Albers *(1)* postulated that the enzyme dissociated into apoenzyme and coenzyme when the pH was lowered below the pH optimum (for hydrolysis), thus reducing its activity. Morton *(18)* obtained no evidence for dissociable organic coenzymes of alkaline phosphatases. Sjöström explained the phenomenon on the basis of restoration of the original hydration of the enzyme protein at the normal pH of milk. Further research is needed to elucidate the nature of the pH effect and the influence of rapidity of pH change in relation to milk phosphatase.

Effect of magnesium ion. Purified preparations of milk phosphatase are activated by several divalent metals, including calcium and magnesium *(12, 18)*. Because milk itself was used as a source of phosphatase in the present investigation, it was considered unlikely that calcium ion could be a limiting factor in the activity determinations. To establish if added magnesium ion would result in increased hydrolysis, the phosphatase activity of individual and pooled milks was determined, under optimum conditions, when two levels of this ion were present in reaction mixtures containing combinations of different buffers and substrates. Table 3, representative of the data obtained, shows the results for an individual milk. The 0.001 *M* magnesium ion did not increase substrate

TABLE 3
Effect of magnesium ion on phosphatase activity

Buffer	Substrate	Concentration		Per cent of original phosphatase activity
		$MgCl_2$	$MgSO_4$	
		(*M*)	(*M*)	
Barium borate-hydroxide	Sodium β-glycerophosphate	0.001	——	99.6
		0.01	——	84.6
Barium borate-hydroxide	Disodium phenyl phosphate	——	0.001	101.8
		——	0.01	81.3
Veronal	Sodium β-glycerophosphate	0.001	——	99.7
		0.01	——	79.4

hydrolysis and at the 0.01 *M* level the enzyme was inhibited. It is apparent that the natural magnesium ion concentration of milk was not a limiting factor in the methods used in this investigation.

SUMMARY

Optimum conditions for measuring the hydrolysis of disodium phenyl phosphate by alkaline phosphatase in raw milk included a 0.02919 *M* substrate concentration and a hydrolysis period of 20 minutes at 38.5° C. and pH 10.32 ± 0.02. These conditions differ from those specified in the Sanders and Sager method for determination of phosphatase activity in unheated milk.

Some properties of the enzyme, as it occurs in raw milk, were studied. The pH optimum of the catalyzed reaction increased with increasing concentrations of disodium phenyl phosphate. Where the substrate was sodium β-glycerophosphate, increased substrate concentration did not shift the pH optimum of 9.60 ± 0.02. Michaelis constants were 0.00340 *M* and 0.00560 *M* for disodium phenyl phosphate and sodium β-glycerophosphate, respectively. The energy of activation of the hydrolysis of disodium phenyl phosphate was approximately 9,000 cal / mole. In the absence of substrate, phosphatase was most stable at approximately pH 6.7. A slow decrease in the pH of raw milk resulted in less enzyme inactivation than a rapid decrease. Furthermore, the extent of subsequent restoration of phosphatase activity was greater at the normal pH of milk than at a higher pH. Added magnesium ion did not increase phosphatase activity under the experimental conditions.

ACKNOWLEDGMENTS

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LIPASE SYSTEMS USED IN THE MANUFACTURE OF ITALIAN CHEESE. II. SELECTIVE HYDROLYSIS[1,2]

W. J. HARPER

Department of Dairy Technology, The Ohio State University, Columbus, and Ohio Agricultural Experiment Station, Wooster

Crude lipase preparations selectively hydrolyzed, at different rates, individual fatty acids from triglycerides. Pancreatic lipase liberated C-12 fatty acids or higher, that from Aspergillus niger released the lower ones, and milk lipase released both lower and higher acids. Lipases used in manufacturing Italian cheese hydrolyzed much butyric acid which varied with the enzyme source. Those from the same gland but from different animals varied in their lipolytic activity. *Editor.*

The proportions of individual free fatty acids in ripened cheese are known to influence its flavor characteristics. In certain cheeses, such as Romano and Provolone, where an active lipase is added to the milk prior to coagulation, any difference in selectivity in the hydrolysis of specific fatty acids from milk fat would influence flavor. Indirect evidence of selective hydrolysis has been indicated in studies which showed that the rate of butyric acid liberation in both Provolone and Romano cheese was related to the animal source of the lipase preparation *(4, 6)*. More direct evidence of selective hydrolysis by lipases has been reported recently *(1, 7, 9, 11)*.

Shipe *(11)* found that different ratios of butyric and caprylic acids were liberated from equimolecular mixtures of tributyrin and tricaprylin by lipases of *Aspergillus niger* and *Penicillium roqueforti*. Wilcox *et al. (12)* demonstrated that different ratios of lower fatty acids were liberated when various microorganisms hydrolyzed milk fat. Exploratory research on lipases used in Italian cheese manufacture revealed differences in the relative amounts of butyric acid in the fatty acid fraction after lipolysis of milk fat by these lipases *(1)*.

This paper presents a continuation of studies on the selective hydrolysis of fatty acids. Such information should be valuable both in adding to the knowledge of the cheese-ripening process and as a means of evaluating lipase preparations for cheese manufacture and other food uses.

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EXPERIMENTAL PROCEDURE

The selective hydrolysis of fatty acids from fat by the various lipase systems was determined by measuring the rates of hydrolysis of synthetic triglycerides, and by determining the differences in the end-products of milk fat hydrolysis. The previous study in this series showed differences in 11 lipase systems used in Italian cheese manufacture *(3)*.

Based on the results of that study, five of the same enzyme preparations were studied. They were: (*a*) domestic purified calf rennet paste, (*b*) imported crude kid rennet paste, (*c*) calf oral glandular preparation, (*d*) kid oral glandular preparation, and (*e*) lamb oral glandular preparation. In addition, milk lipase, *Aspergillus* lipase, and hog pancreatic lipase were studied.

Methods of determining triglyceride hydrolysis. The reaction mixture was analyzed by chromatographic techniques. The extent of hydrolysis was determined by direct extraction of the total free fatty acids on a buffered silica gel column *(5)*, the extraction of a 5- to 10-ml. sample being made with 250 ml. of 5% butanol in chloroform.

The mole per cent butyric acid was measured by buffered silica gel chromatography *(2)*.

Buffers. The buffers were phosphate at pH 6.6 (0.2 *M*) and phthalate at pH 5.3 (0.2 *M*) (pH levels of milk and cheese, respectively). The strength of the buffers was adjusted to a final concentration of 0.1 *M*.

Substrates. All of the synthetic triglycerides were obtained from Eastman Chemical Company, and were used as the substrates without further purification. The insoluble substrates were emulsified with the buffer in a Waring-type blender. Time of mixing was varied, so that the globule size of the different glycerides was approximately ten microns. The various triglycerides were present in the final reaction mixture in concentrations of 0.1 *M*.

Incubation temperatures were maintained at 32° C.±0.5° C. The incubation time was selected so that the rate of hydrolysis was linear with respect to time. Incubation times reported in this study varied from 0.5 of an hour to 3 hours. The enzymatic activity was proportional to the enzyme concentration, and substrate was always present in excess.

Phospholipase activity of preparations. In initial trials with tributyrin, lecithin (soybean) was used as an emulsifying agent. The tributyrin was emulsified with pH 6.6 buffer and 0.1% lecithin. Results revealed that some of the enzyme preparations contained phospholipases which hydrolyzed fatty acids from the phospholipid molecule. Chromatographic analysis *(2)* revealed that about ten to 20 mole per cent of free acids were "nonbutyric" fatty acids, when the crude kid rennet paste or milk supplied the lipase source. Butyric acid only was found when the glandular enzyme preparations were used. Since some of the preparations contained phospholipase activity, lecithin was not used in further experiments. Samples were shaken during incubation with a Burrell "wrist-action" shaker.

Hydrolysis of synthetic triglycerides. The activity of the various enzyme preparations was determined on tributyrin, tricaproin, tricaprylin, and tripal-

TABLE 1

Hydrolysis of triglycerides by different commercial lipase preparations [a]

Enzyme preparations	Relative hydrolysis					
	Tri-butyrin	Tri-caproin	Tri-caprylin	Tri-caprin	Tri-laurin	Tri-palmitin
	(%)					
Domestic calf rennet paste	33	58	17	100	58	65
Imported kid rennet paste	100	68	18	91	12	24
Glandular oral calf preparation	100	66	5	88	24	19
Glandular oral kid preparation	100	31	87	80	32	21
Glandular oral lamb preparation	53	100	55	60	15	8

[a] Average of three trials.

mitin. All studies on the synthetic glycerides were conducted at pH 5.3, which is the average pH level of Italian cheese. The relative hydrolysis of the different triglycerides by the various enzyme preparations is shown (Table 1).

Results are presented as per cent of the glyceride which was hydrolyzed at the fastest rate by each preparation (the glyceride hydrolyzed at the fastest rate was arbitrarily set at 100%). Generally, tributyrin was more rapidly hydrolyzed than the other triglycerides. However, there was a definite difference in the relative rates of hydrolysis by the different preparations. For example, although the glandular calf and kid preparations hydrolyzed tributyrin more rapidly than other glycerides, the kid preparation hydrolyzed tricaprylin 17 times as fast as did the calf preparation. The imported rennet paste and glandular calf preparation, however, were quite similar in the rates at which they hydrolyzed the various triglycerides.

Method of determining selectivity of milk fat hydrolysis. The incubation temperature, enzyme sources, and buffers were the same as those used for the triglyceride study. The milk fat substrate was in the form of 20% cream which had been homogenized at 2,500 p.s.i. and pasteurized at 170° F. for 30 minutes. Analysis for butyric and total free fatty acids was made by the chromatographic method previously cited *(2)*. Caproic, caprylic, and capric acids were determined by the method of Ramsey and Patterson *(9)*.

Selective liberation of butyric acid from milk fat. The selective liberation of butyric acid from milk fat by various lipase preparations was reported previously *(1)*. These trials, which were conducted under more controlled conditions, confirmed the earlier work for butyric acid. Results revealed that the ratio of butyric to total acid was not always a constant throughout the incubation period. Therefore, it was necessary to establish experimentally when this ratio was constant for each different enzyme preparation. The effect of time on the ratio of butyric and higher acids liberated is illustrated by the representative data in Figure 1, which show the ratio of butyric acid per total acids liberated for five of the enzyme preparations. The results, presented as mole per

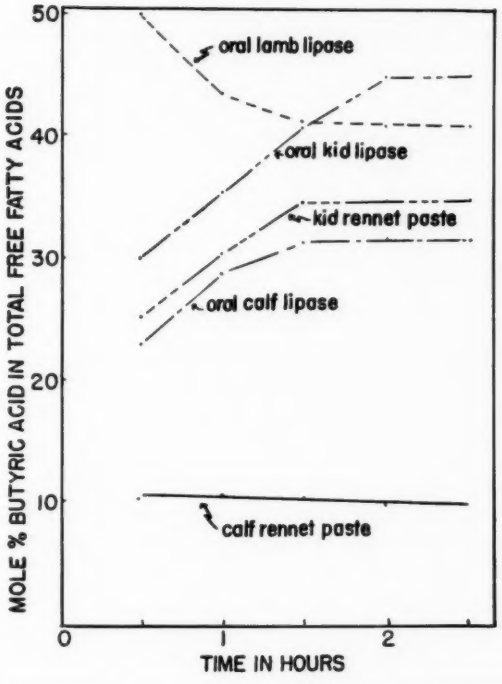

FIG. 1. Effect of incubation time on the relative concentration of butyric acid hydrolyzed from milk fat.

cent butyric acid in the total free fatty acids, showed that in the case of the calf and kid glandular preparations and the kid rennet paste, the butyric acid is released at a faster rate than the higher acids after the first half-hour, but that the relative rate is constant after two hours. The lamb lipase product produced more butyric acid initially during the first 90 minutes, then the ratio remained relatively constant for the next two hours.

The mole per cent butyric acid remained a constant between two and five hours at 37° C. and between five and 20 hours at 5° C. The mole per cent of other fatty acids was constant within the same time limits.

Selective hydrolysis of butyric, caproic, caprylic, and capric acids from milk fat. Further experiments were conducted in which the analyses of free fatty acids were extended to include caproic, caprylic, and capric acids. Conditions in these experiments were identical to those previously described, except for the additional analyses. In addition to the five lipase systems previously studied, freeze-dried raw milk, *Aspergillus* and pancreatic lipase were also investigated. Results are presented (Table 2).

Results for butyric acid are within the range reported previously *(1)*. The relative amounts of the other free acids also varied according to the enzyme

TABLE 2

Selective liberation of individual fatty acids from milk fat by eight different lipase preparations

Lipase source	Butyric	Caproic	Caprylic	Capric	Lauric and higher
	(%)[a]				
Imported crude kid rennet paste	32.8	11.3	7.1	11.8	33.6
Domestic purified calf rennet paste	10.7	3.1	trace	trace	86.5
Calf oral lipase	36.7	8.9	4.8	10.7	39.0
Kid oral lipase	44.4	15.2	7.6	12.3	21.5
Lamb oral lipase	48.1	8.6	14.2	9.3	19.8
Aspergillus lipase	43.1	18.9	20.2	17.5	trace
Milk lipase	13.5	8.2	10.2	8.7	60.0
Pancreatic lipase	8.4	2.1	trace	trace	89.1

[a] Values expressed as per cent of total free fatty acids (on microequivalent basis). Average of five trials. Reaction time not in excess of three hours at 35° C.

source. Pancreatic lipase action resulted in 90 mole per cent of higher fatty acids in the total free fatty acid mixture; whereas, only a trace of these acids was hydrolyzed from milk fat by the *Aspergillus* lipase preparations. Similarly, there is considerable selectivity in the hydrolysis of caproic, caprylic, and capric acids from milk fat. The mole per cent of caproic acid in the total free fatty acids varied from 2.1 to 28.9. The imported kid rennet paste, kid oral lipase, and *Aspergillus* lipase all released more than ten mole per cent caproic acid. Similar variation in amount of caprylic and capric acid present in the free fatty acid mixture was noted.

Some of the preparations are known to contain two or more different lipase enzymes. Schwartz *(10)* demonstrated that the milk lipase system is heterogeneous, and Harper and Gould *(3)* demonstrated two or more lipases present in the three different oral lipase preparations.

Factors affecting selective hydrolysis. Limited studies were made concerning the effect of pH, the temperature of the reaction on the selective hydrolysis of butyric acid from milk fat, and the effect of partial heat inactivation. The calf and kid glandular preparations were chosen for these studies, because they were shown to possess different lipase systems. The calf lipase had pH optima at about 5.3, 6.1, and 7.6; whereas, the kid lipase had pH optima at about 5.9 to 6.2 and about 8.6 *(3)*.

Studies were made at pH 5.3 and 6.6 since these values represent the pH of cheese and milk, respectively. The samples were adjusted to their respective pH levels prior to heating. Temperature used for inactivation was selected as based on temperatures used in treatment of Provolone curd. Results in Table 3 show the effect of partial heat inactivation (140° F. for 15 and 30 minutes), reaction temperatures, and pH on the selective liberation of butyric acid.

Results show that the kid lipase hydrolyzed the same relative amount of butyric acid from the fat regardless of the pH, reaction temperature, or degree of heat inactivation. However, the calf lipase showed different butyric acid values at pH 5.3 and pH 6.6. The mole per cent butyric acid (at pH 5.3) was

TABLE 3

Effect of various environmental factors on the selective hydrolysis of butyric acid from milk fat [a]

Time heated at 60° C.	pH 6.6			pH 5.3		
	Heat inactivation of lipase	Butyric acid		Heat inactivation of lipase	Butyric acid	
		5° C.	35° C.		5° C.	35° C.
(*min.*)	(%)	(*mole* %)		(%)	(*mole* %)	
			Kid lipase			
0	0	39.7	39.5	0	38.6	39.4
15	45	38.7	39.3	12	39.7	39.2
30	76	39.2	38.0	20	39.1	39.0
			Calf lipase			
0	0	32.6	34.5	0	37.8	39.8
15	56	31.2	33.6	27	34.7	36.7
30	82	30.7	32.5	48	36.5	35.3

[a] Figures are averages of two trials. Reaction time was two hours at 35° C. and five hours at 5° C.

about the same as for the kid lipase. The data also revealed slight but consistent changes in the relative amount of butyric acid when the reaction temperature was changed or when some heat inactivation had occurred. Differences in the two enzyme preparations might be related to differences in the lipases present in the two preparations, to differences in inhibitors present, or to the action of other enzymes. The last possibility cannot be ignored, since lower fatty acids, especially butyric acid, can serve as the substrate for other enzyme systems. If enzyme systems were present in the crude preparations which could utilize the fatty acids, then the results could be an index of the selective utilization of the various fatty acids as well as indicating selective hydrolysis.

DISCUSSION

The selective release of fatty acids from milk fat by different lipase preparations has been demonstrated in this study. However, interpretation of these findings in regard to the reason or mechanism of the selection is not possible at this time. All of the lipase preparations were crude materials containing numerous other materials in addition to lipases. The possible presence of other enzymes, inhibitors, or activators could conceivably influence the relative proportions of the various acids in the total free fatty acid mixture.

In addition, the selective utilization of specific acids might influence the results. However, analyses revealed the complete absence of any detectable concentration (less than 0.1 mg./100 ml. milk) of Beta-keto acids, indicating lack of Beta-oxidation of fatty acids. However, this is insufficient evidence to justify final conclusions.

Since the completion of this work, Mattson *(8)* has reported evidence to show that crude pancreatic lipase is substrate specific in its action, in regard to the position of the fatty acid on the triglyceride molecule. In view of this report, it is conceivable that the selectivity of hydrolysis from a complex fat such as

butter fat is an actual indication of true substrate specificity. Proof of this hypothesis will depend on further investigation with relatively pure lipase preparations.

The findings of this study have considerable practical significance. They provide a basis for a sound explanation of the failure of some lipase systems to produce satisfactory flavor in Italian cheese. In addition, the findings suggest a tool for use in evaluating lipase systems for flavor production. By knowing the end-products desired, analyses can be made on the acids released from the substrate to be used. By selecting a preparation that produces free fatty acids in similar proportion to that desired, a manufacturer can eliminate guesswork in his lipase selection. Similarly, lipase selections can be made for other food products, such as in the manufacture of milk powder for milk chocolate manufacture.

The fact that heat treatment had little if any effect on the selectivity of fatty acid hydrolysis is significant to the manufacture of Italian cheese, especially Provolone. This means that the cheese manufacturer will obtain the same type of lipase action in Provolone as in Romano. Therefore, he will need to correct only for loss of total lipase activity.

SUMMARY

Various crude lipase preparations were shown to hydrolyze triglycerides at different rates. Analysis for free fatty acids released by the hydrolysis of milk fat revealed that these same lipase preparations were selective in the individual free fatty acids hydrolyzed from the same substrate, under the same experimental conditions. The type of acids predominating in the free fatty acid mixture varied with the enzyme preparation. Pancreatic lipase hydrolyzed predominantly fatty acids of C-12 or higher, whereas the *Aspergillus* lipase released primarily the lower ones. Milk lipase released significant concentrations of both higher and lower fatty acids. Lipases used for the manufacture of Italian cheese hydrolyzed relatively high concentrations of butyric acid, which varied with the animal source of the lipase. Lipases from the same gland, but from different animals, produced different relative amounts of free fatty acids.

The use of heat treatment similar to that used for Provolone cheese revealed no effect on selective hydrolysis, and only about 25% loss of lipase activity at the pH of the cheese curd.

The selective hydrolysis of fatty acids from milk fat provides a basis for the selection of lipase preparations for Italian cheese manufacture.

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EFFECT OF NORDIHYDROGUAIARETIC ACID (NDGA) AND OTHER FACTORS ON STABILITY OF ADDED VITAMIN A IN DRY AND FLUID MILKS[1, 2]

D. H. COX,[3] S. T. COULTER, AND W. O. LUNDBERG
Departments of Dairy Husbandry and Agricultural Biochemistry
University of Minnesota, St. Paul

Vitamin A palmitate in glyceryl mono-oleate was moderately stable in air-packed nonfat dry milk solids and appreciably less stable in air-packed nonfat dry milk solids. Its destruction was less in dried milks preheated to 180° F. for 30 minutes than in those preheated to 145° F. Vitamin A in whole milk and in skimmilk gradually deteriorated at 40° F. in dark storage, and it was rapidly destroyed in clear-glass bottles in sunlight. NDGA protected vitamin A in air-packed dry milks and also retarded its destruction in fluid milks. *Editor.*

Fortification of dairy products with vitamin A is increasing. Information about the stability of this added vitamin during storage of the products is meager. It is reported to be unaffected in the ordinary processing of fluid milk *(19)* or in the spray-drying of whole milk *(15)*.

To retard destruction of natural vitamin A, preheat treatments (*13*) and packing with inert gas *(7, 13, 15, 16)* have been found to be effective with some products. Also NDGA, one of several antioxidants that have been found to retard appreciably the rancidification of fats *(12)* and to stabilize vitamin A *(3, 8, 9, 14)*, retards the development of oxidized flavors in dairy products *(2, 11, 17, 18)* and protects natural vitamin A in frozen cream *(11)*.

Appreciable information has been obtained about the production of off-flavors and the destruction of nutrients caused by irradiation of milk. Krukovsky *et al. (10)* have reported that a large loss of vitamin A occurs when milk fat is exposed to diffuse daylight for eight hours.

The objectives in the present study were to study (*a*), the effects of several factors on the stability of added vitamin A in dry and fluid whole milk and skimmilk and (*b*), the effectiveness of NDGA as a stabilizer.

EXPERIMENTAL PROCEDURE

Raw whole milk standardized at 3.5% fat was obtained from the University of Minnesota milk plant supply. Skimmilk was obtained by heating whole milk

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[2] Data presented in this paper are taken from a thesis by D. H. Cox in partial fulfillment of requirements for the M.S. degree, University of Minnesota, 1953.

[3] Present address: Department of Animal Diseases, Georgia Coastal Plain Experiment Station, Tifton, Georgia.

to 100° F., separating, and cooling immediately to 40° F. The milks, in standard ten-gallon milk cans, were heated to temperatures of 145, 160, and 180° F. and held for 30 minutes with continuous stirring. The milks to be dried were condensed in a laboratory condenser to approximately 35% total solids.

In all experiments, vitamin A palmitate was added to the milks at a concentration of 2,000 I.U. per quart (or per original quart, in the case of the condensed milks).

In initial experiments, condensed milk samples were prepared to which vitamin A palmitate was added directly, without a carrier solvent. In samples where NDGA was also used, the NDGA was dissolved in glycerol and added separately. When vitamin A palmitate alone was added directly, its destruction in air-packed dry whole milk and nonfat dry milk solids prepared from such samples was excessively high, and the performance of samples that also contained NDGA was not appreciably better. In all subsequent experiments, therefore, glyceryl mono-oleate (Grade S1097)[4] was used as a carrier for both vitamin A palmitate and NDGA.

NDGA, as a 10% solution in glyceryl mono-oleate, was added to the condensed whole milk at a concentration of 0.037%, based on the weight of the fat, and to the condensed skimmilk at a concentration of 0.0144%, based on the weight of the solids-not-fat. Higher concentrations were also studied, but imparted a definite off-flavor without giving any appreciable added stability; results for higher concentrations, therefore, are not reported. It was not established to what extent off-flavors were due to NDGA, the vitamin A palmitate that was used, or the mono-oleate carrier, either alone or in combination.

NDGA was added to the fluid whole milk at a concentration of 0.005%, based on the weight of the fat, and to fluid skimmilk at a concentration of 0.0016%, based on the weight of the solids-not-fat. The vitamin A palmitate was mixed with the NDGA solution prior to addition to the milks. The same amount of solvent was used in preparing samples to which vitamin A palmitate alone was added.

The fortified condensed and fluid milks were heated to 110° F., homogenized at 2,500 p.s.i., and cooled to 40° F. The condensed milks were dried, using a laboratory spray-drier. The powders were packed in No. 202 tinned cans, and half of the samples nitrogen-packed. The powders were stored at room temperature and at 100° F. When it was desired to increase the moisture level of the powders, samples were spread on parchment paper and exposed to an atmosphere of moist air.

The fluid milks were bottled in amber- and clear-glass bottles and waxed-paper cartons. Some of the samples were irradiated by placing the bottled milks outdoors in direct sunlight for periods of 1.5 and three hours. During this time, the temperature of the milks did not change appreciably, because the temperature of the outside air was about 35° F. All of the bottled milks were stored in the University milk plant cooler, which was maintained at about 40° F.

[4] Supplied by the Glyco Products Company, Inc., Brooklyn, New York.

The absorption spectra of the amber- and clear-glass bottles were obtained by placing pieces of the original glass in a Beckman model DU spectrophotometer and measuring the per cent transmission, using air as the reference.

Vitamin A was determined according to the method of Boyer *et al. (1)*. Thiamin disulfide reducing substances were determined essentially according to the method of Harland and Ashworth *(6)*.

RESULTS

Stability of added vitamin A in dry milks. Results for control samples (vitamin A alone), and samples to which NDGA was added, are given (Tables 1 and 2).

TABLE 1

Influence of NDGA, storage temperature, gas-packing, and preheat treatment on the stability of added vitamin A in dry whole milk

(Concentration of NDGA—0.037% based on weight of fat)

Sample	Preheat temp. (° F.)	Packed in	Storage temp. (° F.)	Per cent remaining months 1	2	3	Av. TDRS[a]
Control	145	Air	72	93.7	87.7	82.3	
NDGA	145	Air	72	105.0	100.0	98.6	0
Control	145	N_2	72	102.0	101.0	95.6	
Control	145	Air	100	86.7	78.6	68.6	
NDGA	145	Air	100	98.4	94.3	80.3	
Control	145	N_2	100	96.4	97.4	91.8	
Control	180	Air	72	103.0	95.3	88.3	
NDGA	180	Air	72	106.0	102.5	102.0	9.7
Control	180	N_2	72	112.0	106.0	93.3	
Control	180	Air	100	96.5	91.3	78.8	
NDGA	180	Air	100	102.0	101.5	95.6	
Control	180	N_2	100	105.6	107.7	95.1	

[a] Thiamin disulfide reducing substances, micrograms cysteine-HCl per 100 mg. of powder.

In general, with the dry whole milk samples (Table 1), the preheat treatment at 180° F. imparted greater stability than treatment at 145° F. This may be related to the production of thiamin disulfide reducing substances, which were formed at the higher temperature.

As expected, packing under nitrogen was effective in retarding the destruction of vitamin A in all samples. The rather marked losses in nitrogen-packed samples that received the 145° F. preheat treatment were probably due to inadequate gas-packing rather than to leakage; this is suggested by the observation that the losses were marked during the first month of storage (while residual oxygen was being depleted), then leveled off in the two succeeding months.

Also to be expected was the greater loss of vitamin A in samples stored at 100° F., compared with those stored at 72° F., in cases where samples had access to air.

With the nonfat dry milk solids (Table 2), increasing the moisture content enhanced the stability of vitamin A in samples that had been preheated at 180° F., but there was no effect in samples preheated at 145° F.

TABLE 2

Influence of NDGA, storage temperature, gas-packing, preheat treatment, and moisture on the stability of added vitamin A in nonfat dry milk solids

(Concentration of NDGA—0.0144% based on weight of solids-not-fat)

Sample	Preheat temp. (° F.)	Packed in	Storage temp. (° F.)	Moisture per cent	Per cent remaining months 1	2	3	Av. TDRS[a]
Control	145	Air	72	3.0	56.3	37.7	34.5	
Control	145	Air	72	4.8	51.3	38.3	27.1	
NDGA	145	Air	72	3.0	100.7	99.6	106.3	0
Control	145	N_2	72	3.0	63.0	59.8	55.0	
Control	145	Air	100	3.0	49.4	27.4	22.0	
Control	145	Air	100	4.8	34.5	28.3	22.0	
NDGA	145	Air	100	3.0	104.0	85.7	86.8	
Control	145	N_2	100	3.0	64.7	50.6	41.7	
Control	180	Air	72	2.8	65.3	40.5	37.3	
Control	180	Air	72	4.8	103.0	64.5	53.1	
NDGA	180	Air	72	2.6	119.0	106.0	104.8	
Control	180	N_2	72	2.8	105.0	100.0	100.0	
Control	180	Air	100	2.8	36.9	25.7	19.7	9.9
Control	180	Air	100	4.8	88.0	47.6	27.6	
NDGA	180	Air	100	2.6	111.0	95.3	96.2	
Control	180	N_2	100	2.8	100.8	91.0	82.3	

[a] Thiamin disulfide reducing substances, micrograms cysteine-HCl per 100 mg. of powder.

NDGA effectively retarded the destruction of vitamin A in all air-packed samples. The effect of NDGA was more apparent than with dry whole milk, probably because the control samples of nonfat dry milk solids showed appreciably greater losses of vitamin A. The beneficial effect of NDGA was less apparent in samples stored at 100° F., and in samples preheated at 180° F.

Stability of added vitamin A in fluid milk. Table 3 summarizes the data on stability of added vitamin A in the two fluid milks; these data are for samples that had been pasteurized at 145° F. for 30 minutes.

With samples that were stored in the dark without previous exposure to sunlight, losses of vitamin A in whole milk were relatively slight; however, they were somewhat greater in skimmilk. Results were the same for all three types of containers.

Irradiation of both the whole milk and skimmilk samples in plain-glass bottles for one and one-half and three hours resulted in marked losses of vitamin A, particularly in skimmilk. Losses were markedly less in the samples stored in amber-glass bottles. With waxed-paper containers the penetration of sunlight apparently was insufficient to influence the results.

NDGA effectively retarded the destruction of vitamin A in all samples except those exposed to sunlight in plain-glass bottles. In the latter, the NDGA appeared to be completely ineffective.

Light transmission of the clear-glass and amber-glass bottles was examined in the range from 200 to 1,000 mμ. With the clear glass, the light transmission increased with increasing wavelength to a miximum of 90% at about 360 mμ and remained at this level up to 1,000 mμ. The amber glass began to transmit appreciable light at about 450 mμ and a maximum of 57% transmission was

TABLE 3

Influence of NDGA, sunlight, and type of container on the stability of added vitamin A in milks

Sample	Type container[a]	Not sunlight-treated Per cent remaining Days in dark 3	6	Sunlight-treated Per cent remaining Hours in sunlight 1.5	3	Days in dark 3	6
			Whole milk				
	A	92.1	88.7	91.2	88.0	84.6	85.6
Control	P	88.7	88.5	84.2	70.9	69.2	68.4
	W	89.5	90.3	100.0	91.2	86.8	87.8
0.005%	A	100.0	100.6	100.0	97.0	99.5	100.1
NDGA	P	99.2	100.0	85.5	68.1	68.1	66.2
	W	99.7	100.2	100.0	100.0	100.1	100.6
			Skimmilk				
	A	79.8	75.4	96.6	93.4	75.8	73.7
Control	P	76.0	71.9	55.5	39.7	35.2	30.2
	W	75.5	73.3	99.4	98.4	83.5	79.4
0.0016%	A	100.1	100.1	99.8	96.5	97.4	99.4
NDGA	P	100.0	100.0	45.5	34.9	33.6	34.4
	W	100.1	100.0	100.0	99.3	98.4	99.5

[a]A—amber-glass bottles; P—plain-glass bottles; W—waxed-paper cartons.

attained at 700 mμ, which gradually decreased to 45% transmission at 1,000 mμ. These observations do not permit any conclusions about whether differences of wavelength or differences of light intensity were more important; probably both factors affected the results.

DISCUSSION

The observation that the added vitamin A is more stable in dry whole milk than in nonfat dry milk solids suggests that the fat in dry whole milk is involved in some way in stabilizing vitamin A. Both chemical and physical factors are probably involved. Natural antioxidants such as tocopherols are known to be present in butterfat, and probably afford protection to the vitamin A as well as the fat. Secondly, when vitamin A is added to dry whole milk by the method employed in these experiments, it is likely that the vitamin A is dissolved in the fat phase. On the other hand, in nonfat dry milk solids, the vitamin A is distributed in more concentrated form, and with more surface exposure on nonfat materials. These factors would be expected to enhance susceptibility to oxidative destruction.

It has been found previously that the resistance of dried milk to oxidative deterioration during storage is enhanced by a preheat treatment that produces free sulfhydryl groups. Also, it is known that the destruction of vitamin A occurs more rapidly in the presence of oxidizing fat through a coupled oxidation reaction. Thus, it was to be expected, as observed here, that a preheat treatment that produced free sulfhydryl groups would protect vitamin A as well as the fat.

This explanation of the effect of the preheat treatment is further substantiated by the observation that the effect was further enhanced by high moisture

content. It has been found previously *(5, 20)* that sulfhydryl compounds, such as thiourea and cysteine, are more effective in retarding rancidity in fats in the presence of water.

It was found that adequate gas-packing under nitrogen effectively retarded the destruction of vitamin A in dry milk. Since dry whole milk is usually gas-packed under nitrogen, antioxidants such as NDGA would be useful primarily as precautionary measures, to protect against improperly sealed containers or inadequate gas-packing. Commercially packaged skimmilk is generally not gas-packed, and the addition of antioxidants to stabilize added vitamin A might be of greater practical importance than with dry whole milk.

The ineffectiveness of NDGA in retarding the destruction of vitamin A in fluid milk by irradiation is consistent with similar results obtained by Doctor and Banerjee *(4)*, in studies of the stabilizing effects of hydroquinone, sodium citrate, and sodium tartrate on vitamin A in ghee.

SUMMARY

When incorporated into milks with glyceryl mono-oleate as a carrier solvent, vitamin A palmitate was moderately stable in air-packed dry whole milk, and appreciably less stable in air-packed nonfat dry milk solids. Destruction was less in dried milks which had received a preheat treatment at 180° F. for 30 minutes, compared with a preheat treatment of 145° F. for 30 minutes, presumably due to the production of free sulfhydryl groups in the former treatment. Adequate gas-packing under nitrogen effectively retarded the destruction of added vitamin A.

Vitamin A in fluid whole milk and skimmilk underwent gradual deterioration during normal storage of the milk at 40° F. in the dark, but was rapidly destroyed if the milks were exposed to sunlight in clear-glass bottles. Such destruction by light was greatly retarded if amber-glass bottles or waxed-paper containers were used.

The antioxidant NDGA was found to be markedly effective in protecting vitamin A in air-packed dry milks, particularly nonfat dry milk solids. It also retarded vitamin A destruction in fluid milks, except in samples exposed to sunlight in clear-glass bottles.

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VOLATILE CARBONYL COMPOUNDS PRODUCED IN SKIMMILK BY HIGH-HEAT TREATMENT

M. E. MORGAN

Department of Animal Industries, Storrs Agricultural Experiment Station, Storrs, Connecticut

AND

D. A. FORSS AND S. PATTON

Department of Dairy Science, The Pennsylvania Agricultural Experiment Station, University Park

Furfural and acetaldehyde are the principal volatile carbonyl compounds generated in skimmilk by high temperatures. None of the aldehydes which could come from a heat-induced reaction between leucine, isoleucine, or valine and the dicarbonyl compounds in raw and heated skimmilk was found in skimmilk heated for 90 minutes at 121° C. When leucine or isoleucine was added to raw skimmilks, 3-methylbutanal or 2-methylbutanal was isolated from distillates of the heated skimmilks. *Editor.*

Amino acids when heated with certain types of dicarbonyl compounds in neutral aqueous solution undergo the so-called Strecker degradation, to yield aldehydes containing one less carbon than the original amino acid *(3, 14)*. Because normal milk contains trace amounts of a variety of free amino acids *(1, 10, 15)* and because high-heat treatment causes the generation of such carbonyl compounds as methyl glyoxal *(6)* and pyruvic acid *(7)*, as well as the probable increase in free amino acids through the hydrolytic effects of the heat treatment on milk proteins, one of the present authors has postulated that a number of flavorful aldehydes may be produced during excessive heat treatment of milk as a result of the Strecker degradation *(12)*. The recent report *(13)*, that unheated milk contains appreciable amounts of alpha-ketoglutaric acid, further supports the contention that the reactants necessary for the Strecker degradation are present. Sufficient free leucine may be present in milk to favor the formation of 3-methylbutanal during heating. A very small quantity of this compound will impart a malty (or caramel) flavor to milk *(5)* and might conceivably be a component of the caramel flavor of high-heat–treated milk.

Detection of carbonyl compounds in heated milk distillates. Three-liter quantities of raw or vacreated skimmilk with and without added amino acids and dicarbonyl compounds were boiled under reflux or autoclaved at 121° C. for various periods. One-liter portions of the latter samples were autoclaved in three two-liter Erlenmeyer flasks covered with inverted 150-ml. beakers. Each

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of the heated samples and a raw control sample were subjected to steam distillation. One hundred ml. of distillate was collected from each liter of sample in an ice-cold receiver in about 15 minutes. Distillates from each of the three liters of similarly treated milk were combined, warmed to room temperature, treated with 30 ml. of 0.2% DNP[1] hydrazine in 2 *N* HCl, and held overnight.

Each distillate was extracted with two 100-ml. portions of a 1:1 carbonyl-free benzene-heptane mixture. The extracts were washed with three 50-ml. quantities of water and then evaporated to dryness in a rotating flash evaporator. The residues were taken up in a minimum of warm benzene-heptane solvent and poured onto 80 × 28 mm. columns of anhydrous magnesium sulfate. The DNP hydrazones were eluted from the columns with additional solvent under nitrogen at from three to five p.s.i., leaving the unreacted DNP hydrazine in a band at the top of the columns. The eluates were evaporated to a convenient volume and made up to 50 ml. with solvent. Then 0.04-ml. of the derivative mixture from each sample, and appropriate quantities of benzene-heptane solutions of DNP hydrazones of known carbonyl compounds, were spotted on Whatman No. 1 paper and chromatographed by the procedure of Huelin *(4)*.

Figure 1 is a tracing of the chromatogram of the carbonyl DNP hydrazones formed in the distillates of the variously treated skimmilks. That raw skimmilk contains appreciable amounts of a volatile carbonyl compound, presumably acetone, is indicated by the large derivative spot from Sample 1. A subsequent chromatogram of dilutions of this preparation yielded but single spots, running with known acetone derivative. The derivatives extracted from the distillate from the skimmilk which had been boiled for three hours gave three faint spots, running with furfural, acetaldehyde, and acetone derivatives, respectively. The apparent loss of acetone during this heat treatment was probably due to inefficient condensation during refluxing. The derivative mixtures from Samples 3, 4, and 5 which had been heated at 121° C. for 30, 60, and 90 minutes, respectively, yielded progressively more intense spots corresponding to furfural and acetaldehyde derivatives; whereas, there was no discernible increase in the acetone derivative. No spots corresponding to isobutyraldehyde or 3-methylbutanal derivatives were detected.

Since none of the higher aliphatic aldehydes was generated during rigorous heat treatment of skimmilk, the treatment of Samples 6, 7, and 8 was designed to determine whether conditions in skimmilk are favorable for the operation of the Strecker degradation during such heat treatment. DL-leucine was added to Samples 6 and 7 at the rate of 0.076-gm. per liter, and 0.2-gm. of sodium pyruvate per liter was added to Sample 6 to provide an excess of dicarbonyl compound. DL-isoleucine was added to Sample 8 at the same rate, and all three samples were autoclaved at 121° C. for 60 minutes. As seen in Figure 1, the derivative mixtures extracted from the distillates of these three samples each gave spots running parallel with the 3-methylbutanal derivative. These spots were barely visible in natural light, but were definitely detectable under ultra-

[1] DNP is used throughout this paper as an abbreviation for 2,4-dinitrophenyl.

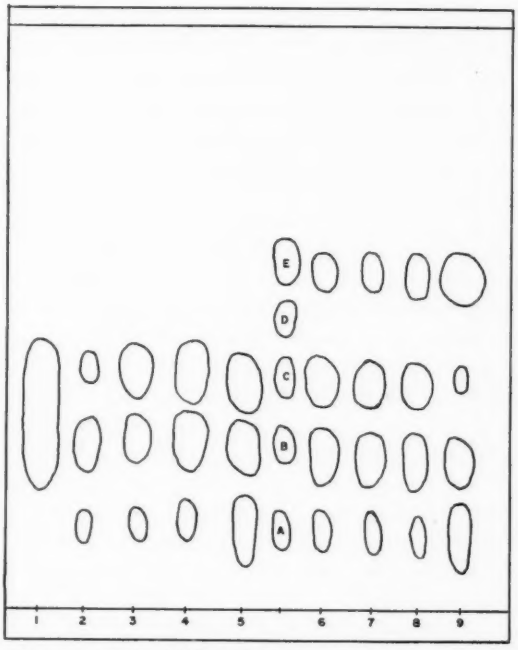

FIG. 1. Chromatogram of the DNP hydrazones of carbonyl compounds distilled from raw skimmilk (1) treated as folows: refluxed three hours (2); autoclaved at 121° C. for 30 minutes (3), 60 minutes (4), and 90 minutes (5); 0.076-gm. DL-leucine added per liter and autoclaved at 121° C. for 60 minutes (6), 0.076-gm. DL-leucine and 0.2-gm. sodium pyruvate added per liter and autoclaved at 121° C. for 60 minutes (7), 0.076-gm. DL-isoleucine added per liter and autoclaved at 121° C. for 60 minutes (8); vacreated, 0.076-gm. DL-leucine added per liter and autoclaved for 90 minutes (9). (A), (B), (C), (D), and (E) are authentic derivatives of furfural, acetaldehyde, acetone, isobutyraldehyde, and 3-methylbutanal, respectively.

violet light (3,600 A). The inclusion of excess pyruvate in Sample 7 apparently had no effect on the generation of pentanal from the added leucine.

Sample 9 came from a lot of skimmilk which had been pasteurized at 162° F. (HTST) and immediately passed through a Cherry-Burrell Vacreator at 175° F.; 0.076-gm. of leucine per liter was added and the sample autoclaved at 121° C. for 90 minutes. The derivative mixture formed in the distillate from this sample gave a barely perceptible spot in the acetone position, and the spots for furfural and acetaldehyde derivatives were nearly identical in size and intensity with those from Sample 5. This indicates that the preliminary vacuum treatment removes most of the acetone normally present in raw skimmilk and makes it doubtful if any acetone is generated during intensive heat treatment. The pentanal derivative spot from this sample was somewhat larger and much more intense than the comparable spot from Sample 6, indicating an increased yield from the Strecker degradation, due to the longer heat treatment.

Isolation and identification of carbonyl derivatives. Because the derivative extract obtained from the raw skimmilk distillate appeared to contain but a single component in rather high concentration, the solvent was removed and the residue twice recrystallized from 80% ethanol. Melting of this derivative was observed at from 126 to 127° C. on a Kofler micro-melting point apparatus. A mixed melting point with authentic acetone derivative (from 126 to 127° C.) revealed no depression.

In order to provide sufficient of the derivatives of the heat-generated carbonyl compounds for fractionation and identification, three ten-liter quantities of skimmilk were prepared. The first was raw skimmilk, and the second and third were skimmilk from the same raw supply, but pasteurized and vacreated as previously indicated. DL-leucine and DL-isoleucine were added to the latter two lots of skimmilk, respectively, at the rate of 0.076-gm. per liter. Although the yield of pentanal from the heated skimmilk containing this amount of amino acid, as estimated from the chromatogram (Sample 9) in Figure 1, appeared to be far less than the theoretical (assuming only a 25% conversion), it was anticipated that sufficient would be formed in ten liters of similarly treated skimmilk to permit isolation and identification. The three lots of skimmilk were autoclaved at 121° C. for 90 minutes in a series of two-liter flasks, as previously described. As before, each lot was steam-distilled immediately after the heat treatment.

The procedure employed in obtaining the derivative mixture from the skimmilk distillates was essentially the same as the procedure outlined above, with one exception. The derivatives formed in the distillates from the heat-treated skimmilk were extracted with three 200-ml. portions of the benzene-heptane solvent; whereas, those in the distillates from skimmilk to which no extra amino acids had been added prior to the heat treatment were extracted exhaustively with carbonyl-free petroleum ether. The derivative residue obtained from the heated skimmilk without added amino acid was dissolved in a minimum of warm benzene-heptane solvent and run into a 120 × 28 mm. column of anhydrous magnesium sulfate. The fast-moving derivatives were eluted as a single fraction with additional solvent. The elution was continued until a faint band moving out of the DNP-hydrazine band had been collected. Several chromatograms of different concentrations of the two fractions thus collected revealed that the fast-moving fraction contained derivatives which ran with authentic derivatives of furfural, acetaldehyde, and acetone. The slow-moving fraction appeared to contain three components in very small quantities. One did not move from the base line, and the second and most distinct ran parallel with known acetol derivative a short distance above the base line. The third gave a faint spot running slightly beyond the second.

Cellulose was dry-packed in a 28-mm. column to a height of 300 mm. The column was washed with approximately one liter of methanol to remove a waxy material contained in the cellulose. Heptane-saturated methanol was then run into the column until the heptane was detected in the effluent. Equilibration

was continued until approximately 200 ml. of this solvent phase had passed through the column. The benzene-heptane solution of the first fraction off the magnesium sulfate column was evaporated to dryness and the derivative residue dissolved in a minimum of methanol-saturated heptane. This solution was run into the cellulose column and more of the same solvent was added until the mixture had separated into two yellow bands, followed by a slower-moving buff-colored band. Elution was continued until the middle portion of each band had been collected. The fractions appeared to be pure when chromatographed on paper, the first, second, and third off the column running parallel with known derivatives of acetone, acetaldehyde, and furfural, respectively.

The solvent was evaporated from the derivative fractions and each was crystallized twice from 80% ethanol. The acetone fraction melted at from 126 to 127° C. and a mixed melting point with authentic acetone derivative (from 126 to 127° C.) revealed no depression. Melting of the yellow needles of the acetaldehyde fraction was complete at 167° C., but sublimation was observed beginning at about 145° C. The dark-red crystals of the furfural fraction melted at 222° C. and, when allowed to cool, some of the derivative recrystallized in yellow needles which melted at 185° C. on reheating. Continued heating at 222° C. or above resulted in complete sublimation of the derivative. Both acetaldehyde and furfural DNP-hydrazones are known to have several melting points, depending on the mode of preparation *(2)*. In order to convert these derivatives to their stable forms, the following sublimation procedure was finally adopted. Crystals of the derivatives were added to glass slides and covered with cover slips supported on three pieces of broken cover slip. The slide containing the furfural derivative was placed on the hot stage at 205° C. and the temperature slowly raised, during which time the derivative sublimed completely onto the raised cover slip and recrystallized into a mass of squares and rectangles. The cover slip was transferred to a clean slide and placed on the hot stage at 210° C. and the temperature raised slowly until melting was observed. The isolated and authentic derivatives and a mixture of equal amounts of these were treated in the above manner and the following melting points, respectively, were observed, 229 to 231° C., 231 to 232° C., and 230 to 232° C. Sublimation of the acetaldehyde derivatives was begun at 145° C. and the temperature slowly raised to 162° C., at which point most of the sublimate had recrystallized. The sublimates of the isolated, authentic, and mixed derivatives melted at 166.0 to 167.5° C., 167 to 168° C., and 166.0 to 167.5° C.

The derivative residues from the distillates from the heated skimmilks to which leucine and isoleucine, respectively, had been added were fractionated on the cellulose column. Preliminary passage through a magnesium sulfate column was not required, because the excess DNP-hydrazine and the slow-moving derivatives previously noted in the benzene-heptane extracts are not extracted by petroleum ether. The first band eluted from the column in the fractionation of each mixture was collected and the succeeding bands which had already been identified were discarded. Paper chromatograms of these two fractions revealed

two principal spots running parallel with one another and with 3-methylbutanal and 2-methylbutanal derivatives. However, neither fraction appeared to be pure, for there was a very faint spot just above the derivative from the milk to which leucine had been added, and a similar spot just below the derivative from the milk containing added isoleucine. Because these trace derivatives ran so close to the major fractions, it was felt that efficient separation by repeated fractionation on the cellulose column could not have been accomplished. Therefore, each of the pentanal derivative fractions was chromatographed [according to the procedure in Reference *(9)*] on sheets of Whatman No. 1 paper which had been washed exhaustively with methanol. After development, the bands of pentanal derivative were readily cut from the adjacent faint derivative bands. After elution with methanol and evaporation to dryness, each derivative was twice recrystallized from 80% ethanol. The pentanal derivative isolated from the heated milk to which leucine had been added, authentic 3-methylbutanal DNP-hydrazone, and a mixture of equal quantities of these each melted at from 121 to 123° C. The pentanal derivative isolated from the heated milk to which isoleucine had been added, authentic 2-methylbutanal derivative, and a mixture of these melted at from 128 to 129° C., 129 to 130.5° C., and 127.5 to 128.5° C., respectively. The faint bands adjacent to the pentanal bands were eluted, but the yield of derivative was insufficient to permit further identification.

DISCUSSION

Herbst and Engel *(3)* have shown that to approach completion in the Strecker reaction between amino acids and dicarbonyls in dilute aqueous solutions requires many hours of heating at 100° C. However, both these workers and Schönberg *et al. (14)* have obtained substantial yields of aldehyde in 30 minutes. In the present work, detectable amounts of the aldehydes derivable from free leucine, isoleucine, and valine were not formed in ten liters of normal skimmilk heated at 121° C. for 90 minutes. The leucines and valine have been detected in protein-free filtrates of skimmilk both by paper chromatographic *(1)* and microbiological *(10)* methods. Assays in the latter report revealed approximately 5γ of leucine and 7.5γ of valine per ml. inspissated skimmilk. Assuming this amount of leucine is present along with sufficient dicarbonyl to promote the reaction, the yield of pentanal from ten liters of skimmilk at 25% of theory would be eight mg., or 25 mg. of the corresponding DNP hydrazone. It would appear that had this amount of pentanal been generated, allowing even as much as a 50% loss during autoclaving, distilling, etc., the presence of the pentanal derivative still should have been detected. Although the corresponding pentanals were detected when extra leucine or isoleucine was added to skimmilk prior to the heat treatment, the low yields were taken as evidence that the Strecker degradation of these amino acids is somewhat inhibited in skimmilk.

The presence of a relatively large amount of acetone in the distillate from raw skimmilk was not unexpected, because Knodt *et al. (8)* had detected acetone

bodies in milk from apparently normal cows. The apparent difference in the amounts of acetone recovered from raw and heat-treated skimmilks is probably due to losses incurred during the autoclave treatment of the skimmilk in unsealed containers. This might also be taken to imply concurrent loss of any traces of the higher aldehydes which may have been formed from the leucines or valine. However, the absence of these has been confirmed in a separate trial, in which skimmilk was heated at 121° C. for 30 minutes in sealed evaporated milk cans.

In the present study, acetaldehyde and furfural were the principal heat-generated carbonyl compounds detected in several different samples of skimmilk in two different laboratories. Keeney *et al. (6)* obtained presumptive evidence that acetaldehyde is formed during high-heat treatment of skimmilk. They have postulated that it is an intermediate in the decomposition of methyl glyoxal to acetic acid. This compound could be generated in several ways. Because alanine is presumably one of the more predominant free amino acids in milk, it is quite possible that some acetaldehyde arises through a Strecker degradation of this amino acid. Any other possible degradations involving pyruvic acid and such free amino acids as glutamic acid, glycine, serine, and aspartic acid, would yield additional alanine which, in turn, could be converted to acetaldehyde. While the amounts of individual carbonyl compounds generated from the various amino acids may be small, as appears to be the case with the leucines and valine, the sum total of the generated alanine may be sufficient to yield considerable acetaldehyde.

Furfuryl alcohol and hydroxymethylfurfural have been isolated from rigorously heated skimmilk *(11)*. Control experiments employing simplified casein-carbohydrate systems indicated that the formation of furfuryl alcohol requires the intact lactose molecule; whereas, hydroxymethylfurfural is formed from glucose and galactose, as well as lactose. The present finding, indicating that a considerable amount of furfural is generated during the heat treatment of skimmilk, suggests that this compound may be an intermediate in the formation of furfuryl alcohol. Furfural could well be a primary product in the degradation of lactose, and is partially converted to furfuryl alcohol under the influence of the strong reducing conditions existing in heated milk.

SUMMARY

Furfural and acetaldehyde are the principal volatile carbonyl compounds generated in skimmilk by high-heat treatment. None of the aldehydes which could result from a heat-induced reaction between the free leucine, isoleucine, or valine and the dicarbonyl compounds present in raw and heat skimmilk was detected in skimmilk heated as long as 90 minutes at 121° C. When leucine or isoleucine was added to skimmilks prior to heat treatment, 3-methylbutanal or 2-methylbutanal was isolated from the distillates of the heated skimmilks. The apparent low yield of these compounds suggests that the Strecker degradation may be somewhat inhibited in a medium such as skimmilk.

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ORGANIC ACID PRODUCTION BY PROPIONIBACTERIA. I. EFFECT OF STRAINS, pH, CARBON SOURCE, AND INTERMEDIATE FERMENTATION PRODUCTS[1]

I. S. EL-HAGARAWY, W. L. SLATTER, AND W. J. HARPER
Department of Dairy Technology, The Ohio State University, Columbus

Effects of strain, pH, source of carbohydrate, and intermediate fermentations on propionic and acetic acid production were studied with ten strains of Propionibacterium shermanii. Sodium lactate stimulated acid production for all strains, reaching a maximum in two days, as compared to eight days for lactose. The lactate medium yielded five to six times more total acids than the succinate, and twice more than the pyruvate. It was suggested that lactate may be the precursor of the propionate. *Editor.*

Many workers have found that propionibacteria have a major role in the development of the characteristic flavor of Swiss cheese *(3, 6, 13, 17)*.

Krett and Stine *(14)*, using a chromatographic technique, found that good-flavored Swiss cheese contained relatively large amounts of acetic and propionic acids, with little or no butyric acid and a small amount of higher acids. Poor-grade Swiss cheese contained larger amounts of butyric acid in relation to propionic and acetic acids.

Although the formation of propionic and acetic acids in Swiss cheese has been attributed to the propionic acid bacteria, little information is available concerning the factors influencing the formation of these compounds by this group of organisms.

This investigation was undertaken to study the effect of strains, pH, carbon source, and intermediate fermentation products on propionic and acetic acid production by *Propionibacterium shermanii*.

EXPERIMENTAL PROCEDURE

Cultures employed in this investigation consisted of ten strains of *Propionibacterium shermanii*, three of which (I.S.C., H., and M) were obtained from the Ohio Swiss Cheese Association, Sugarcreek, Ohio, and seven (62, 333, 9614, 9615, 9616, 9617, and commercial) from the Agricultural Research Service, U. S. Department of Agriculture. All organisms had been isolated either from starters used in Swiss cheese manufacture or from Swiss cheese.

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Organisms were maintained in a broth medium of the following composition: 1.0% peptone, 1.0% tryptone, 0.5-% lactose, and 0.5-% yeast extract. The medium was adjusted to pH 7.0 and autoclaved at 15 p.s.i. for 20 minutes. Special media were prepared by substituting various compounds for lactose in the broth medium. All cultures were incubated at 30° C.

Organisms were counted by the standard plate method *(1)* as modified by Kamber *et al. (12)*. The cultures were analyzed for acetic and propionic acids after incubation at 30° C. for varying lengths of time, using a direct silica gel column chromatographic technique *(10)*.

EXPERIMENTAL RESULTS

Characteristic acid production of P. shermanii. In the study of differences in propionic and acetic acid production by *P. shermanii*, ten strains were used. Since the results were essentially the same for all trials, only the data of a typical trial are presented (Table 1). These reveal the relationships between maximum number of organisms, maximum production of propionic and acetic acids, and

TABLE 1

Maximum growth and acid production of ten strains of P. shermanii[a]

Strain	Maximum number of organisms[b]	Age of culture at maximum number	Maximum acid production		Age of culture at maximum acid production
			Propionic	Acetic	
	(1×10^6/ml.)	*(days)*	*(ml.)*[c]	*(ml.)*[c]	*(days)*
9616	170	9	11.5	4.1	9
9615	660	6	11.0	5.6	9
I. S.C.	1,100	2	10.6	4.0	9
M	1,600	2	10.1	5.3	9
333	450	4	10.0	6.8	9
9614	800	9	9.9	5.4	9
H	730	4	9.1	4.7	4
9617	1,300	6	8.9	3.3	4
62	900	4	8.7	5.4	9
Commercial	8	2	—	1.3	12

[a] Results of a typical trial.
[b] Standard plate count per ml.
[c] Values expressed as ml. N/100 base/4 ml. culture.

the period of incubation at which the maximum number of organisms and maximum concentration of these acids occurred. All cultures were incubated for 12 days.

All cultures displayed high activity, with the exception of the strain designated as commercial. This strain was definitely atypical in comparison to all others studied, and was disregarded in the comparisons made. Results reveal wide differences in the age of culture in relation to maximum number of viable organisms. Of the nine cultures, two reached maximum numbers in two days, three in four days, two in six days, and two in nine days. There was no relationship between the time at which maximum number of organisms occurred and the time at which maximum concentration of acid was obtained. Seven of nine strains producted maximum acid in nine days; whereas, two had maxi-

mum acid production at four days. In one of these, the maximum number of organisms also occurred at four days (Strain H) and in the other, the maximum number of organisms occurred at six days. There was no direct relationship between the number of organisms and the amount of acid produced. For example, Strain 9616 produced more propionic acid than Strain M, with one-ninth as many viable organisms in the culture.

Effect of additives to medium. In order to study the effects of carbohydrates on the growth and acid production of *P. shermanii*, the lactose in the standard broth was either increased to 1.0% or replaced by 1.0% glucose. All strains of *P. shermanii* were studied during a nine-day incubation period. Representative results are presented (Table 2) for two strains.

Response in numbers of organisms to an increase in carbohydrate concentration was faster with Strain 62; whereas, the response in acid production was slower with this strain than with Strain 9616. Increasing the amount of carbohydrate to 1.0% (both lactose and glucose) resulted in a marked increase in total acid production at both the six- and nine-day periods. With Strain 62, the increase in acid production was approximately 111% after nine days of incubation with 1.0% lactose; whereas, with Strain 9616 it was 60%. Amounts of acid produced from both 1.0% lactose and 1.0% glucose were almost equal after six days of incubation.

Increase in acid production, which might be expected from the use of glucose as based on the literature *(5, 11)* was not obtained in this study. Among the factors which might explain these results are those suggested by Spiegelman *(18)* which are, *(a)* variation in penetration of the adaptive substrate into the cell, *(b)* formation by existent enzyme systems of special intermediate products unique in the metabolism of the adaptive substrate, *(c)* variation in time required to raise the concentration of existent cofactors to the levels required for the metabolism of the adaptive substrate, and *(d)* formation of an enzyme system in response to the adaptive substrate.

In another experiment, a comparison was made of the effect of lactose and sodium lactate on the growth and acid production of five strains of *P. shermanii*. Concentration of both the lactose and sodium lactate in the medium was 1.0%. The results are presented (Table 3).

The lactate greatly stimulated the acid production of all strains, with maximum acid production being at two days, in contrast to eight days for the lactose media. In general, the total acid production at two days in the lactate media approximated that produced at eight days in the lactose media. Whereas the acid production in the lactose media increased during the total incubation period, that in the lactate media increased sharply to a maximum and then decreased.

The pH of the lactose medium dropped markedly during incubation; whereas, the pH of the lactate medium remained the same, or increased. The drop in pH could not be related to the growth of the culture. Regardless of the drop in pH during incubation, the number of organisms in the lactose medium was the same or higher than that in the lactate medium.

TABLE 2

Effect of lactose and glucose on acid production and growth of P. shermanii

Incubation period	Carbohydrate medium	Strain 62				Strain 9616			
		No. of org/ml[b]	pH	Propionic Acid	Acetic Acid	No. of org/ml[b]	pH	Propionic Acid	Acetic Acid
(days)		*(1×10^6)*		*(ml.)*[a]	*(ml.)*[a]	*(1×10^6)*		*(ml.)*[a]	*(ml.)*[a]
2	0.5% lactose	1,010	5.65	2.60	1.00	90	5.20	4.30	2.30
	1.0% lactose	1,170	5.70	2.70	1.10	210	5.05	6.30	4.00
	1.0% glucose	512	5.60	2.70	1.00	56	5.35	3.60	1.45
4	0.5% lactose	800	4.65	10.91	5.20	420	4.55	11.00	4.80
	1.0% lactose	39	4.85	11.60	6.00	610	4.40	20.20	18.60
	1.0% glucose	600	4.60	8.96	5.80	240	4.42	17.80	6.74
6	0.5% lactose	1,000	4.65	14.80	6.00	660	4.52	13.50	4.60
	1.0% lactose	350	4.70	21.00	7.60	540	4.35	20.55	10.25
	1.0% glucose	440	4.40	20.45	8.50	600	4.32	22.80	9.10
9	0.5% lactose	200	4.75	11.72	6.20	70	4.75	14.05	6.95
	1.0% lactose	60	4.65	29.01	8.95	80	4.45	23.40	11.00
	1.0% glucose	110	4.40	25.50	10.65	65	4.48	18.20	10.00

[a] Values expressed as ml. N/100 base/4 ml. culture.

[b] The initial number of organisms was: Strain 62, 35×10^6/ml.; strain 9616, 48×10^6/ml.

TABLE 3

Effect of lactose and sodium lactate on the growth and acid production of five strains of P. shermanii

Strain	Carbon source	Incubation period in days											
		2 days				4 days				8 days			
		pH	Propionic Acid	Acetic Acid	No. of org/ml	pH	Propionic Acid	Acetic Acid	No. of org/ml	pH	Propionic Acid	Acetic Acid	No. of org/ml
			(ml.)[a]	*(ml.)*[a]	*(1×10^6)*		*(ml.)*[a]	*(ml.)*[a]	*(1×10^6)*		*(ml.)*[a]	*(ml.)*[a]	*(1×10^6)*[a]
H	Lactose	5.20	6.50	3.90	400	4.48	14.95	7.50	900	4.45	22.15	10.15	8
	Lactate	6.20	22.20	13.95	1,200	6.23	14.80	9.10	910	6.25	17.30	10.50	10
333	Lactose	5.80	3.70	2.70	1.8	4.72	12.00	7.70	800	4.50	21.40	13.25	10
	Lactate	6.28	22.80	12.80	40	6.35	16.20	9.90	1,000	6.35	19.35	1.00	10
9617	Lactose	5.75	3.80	2.55	210	4.70	12.90	5.75	1,000	4.50	23.95	10.90	10
	Lactate	6.27	20.20	10.35	160	6.49	16.10	9.35	1,000	6.55	18.65	10.25	10
62	Lactose	5.40	4.60	3.00	120	4.70	11.00	7.15	40	4.55	17.80	10.55	10
	Lactate	6.25	18.30	8.40	120	6.50	16.20	9.60	42	6.70	17.80	10.20	10
9614	Lactose	5.05	6.80	4.20	10	4.50	17.70	9.80	16	4.40	22.60	11.45	10
	Lactate	6.28	17.00	9.50	7	6.30	14.20	10.10	8	6.30	17.85	10.40	10
All strains													
Av.	Lactose		5.08	3.25			13.70	7.60			21.60	11.25	
Av.	Lactate		20.05	11.00			15.50	9.60			18.20	10.50	

[a] Values expressed as ml. N/100 base/4 ml. culture.

Further studies were conducted to determine the effect of certain metabolites such as pyruvate and succinate on the growth and acid production of two strains of *P. shermanii*. In this experiment, the basic medium was modified by substituting the 0.5% lactose with 1.0% concentration of sodium lactate, pyruvate, or succinate. Incubation periods were one and two days. Both initial and terminal pH levels were similar for all media and the number of organisms produced was nearly the same. Results are shown (Table 4).

TABLE 4

Effect of lactate, pyruvate, and succinate on acid production of P. shermanii

Strain	Carbon source	Period of incubation in days			
		1		2	
		Propionic	Acetic	Propionic	Acetic
	(1%)	*(ml.)*[a]	*(ml.)*[a]	*(ml.)*[a]	*(ml.)*[a]
H	Lactate	4.75	2.75	17.55	9.55
	Pyruvate	2.60	2.35	8.20	12.75
	Succinate	2.70	1.25	3.30	1.50
333	Lactate	3.25	2.00	10.10	10.30
	Pyruvate	2.00	1.55	8.15	13.55
	Succinate	2.95	1.55	3.95	2.35

[a] Values expressed as ml. N/100 base/4 ml. culture.

Data reveal that after two days of incubation, the organisms in the lactate medium yielded from five to almost six times as much total acids as was produced in the medium containing succinate, and two times as much as in that containing the pyruvate. From three to four times more total acid was produced in the pyruvate medium than in that containing succinate.

The fact that the medium containing succinate yielded less acid than that containing pyruvate or lactate was unexpected, because it was thought succinate was the logical precursor for the propionic acid formation *(5, 11)*. The more rapid rate of propionic and acetic acid production in lactate medium suggests that under the conditions of these studies lactate is possibly the more immediate and major precursor of the propionate. The fact that the rate of propionic acid production in pyruvate medium is closer to the rate of its production in the lactate, than in the succinate, medium also supports this suggestion. Werkman and Wood *(19)* have indicated that with other propionic bacteria, propionate may be formed through dehydration of lactate to acrylate, which is then reduced to propionate. Leaver and Stjernholm *(15)* have reported that succinate is not the major precursor of propionic acid by this species of organisms.

Effect of initial pH. The effect of varying the initial pH of 1.0% lactate broth on the growth and acid production of five strains of *P. shermanii* is shown (Table 5). The pH values of 6.6, 5.8, 5.4, and 5.2 were investigated. Four of the five strains studied grew better at the higher pH levels. Generally, a similar amount of acid was produced at each pH level.

The original pH had little apparent effect on bacterial numbers of Strain 62 at the various pH levels employed. In each comparison the largest number

TABLE 5

Effect of initial pH on number of organisms and acid production of five strains of P. shermanii

Strain	Incubation period	No. of org.[b]	Propionic Acid	Acetic Acid	No. of org.[b]	Propionic Acid	Acetic Acid
	(days)	*(1×10^6)*	*(ml.)*[a]	*(ml.)*[a]	*(1×10^6)*	*(ml.)*[a]	*(ml.)*[a]
		Initial pH—5.2			Initial pH—5.4		
62	0	10	0.00	0.00	10	0.00	0.00
	2	1,250	11.30	6.30	1,100	16.65	9.20
	4	4	16.95	10.35	0.70	17.80	10.70
9614	0	75	0.00	0.00	75	0.00	0.00
	2	0.20	7.55	3.60	6	16.90	8.70
	4	0.50	18.15	10.80	10	19.10	10.90
333	0	20	0.00	0.00	20	0.00	0.00
	2	7	0.70	1.10	8	7.00	4.10
	4	0.01	19.40	9.65	2	20.15	10.75
H	0	8	0.00	0.00	8	0.00	0.00
	2	4	16.55	11.95	25	16.35	12.90
	4	20	16.20	8.70	30	16.35	8.75
9617	0	60	0.00	0.00	60	0.00	0.00
	2	6	2.20	2.70	300	12.85	6.65
	4	690	14.80	8.65	470	15.30	8.95
		Initial pH—5.8			Initial pH—6.6		
62	0	10	0.00	0.00	10	0.00	0.00
	2	1,670	17.20	9.80	1,300	17.25	9.70
	4	2	18.05	10.95	6	18.30	8.60
9614	0	75	0.00	0.00	75	0.00	0.00
	2	33	19.25	10.05	40	18.25	10.15
	4	27	19.25	11.10	80	18.60	10.35
333	0	20	0.00	0.00	20	0.00	0.00
	2	10	16.95	9.60	250	17.20	9.90
	4	8	19.85	8.80	50	22.50	13.55
H	0	8	0.00	0.00	8	0.00	0.00
	2	150	15.15	12.45	350	16.45	12.80
	4	400	16.40	8.05	600	17.50	8.60
9617	0	60	0.00	0.00	60	0.00	0.00
	2	500	17.70	8.65	130	15.05	9.40
	4	660	14.95	8.45	280	16.05	9.30

[a] Values expressed as ml. N/100 base/4 ml. culture.

[b] Standard plate count.

occurred on the second day and the total count was approximately the same at each pH level. Strains 9614 and 333 decreased in numbers during the four-day incubation period at pH levels 5.2, 5.4, and 5.8. Decrease in numbers was most marked at the lower pH levels. Strains H and 9617 increased in numbers at all pH levels, with increase being greatest at the higher pH levels. Strain H had the highest count at pH 5.8 in four days.

Concentrations of propionic and acetic acid were markedly higher at two days in the cultures at pH 6.6 than at pH 5.2, with the exception of Culture H. However, at pH 5.4 the acid production was almost as high as at pH 6.6. At the end of four days, acid concentrations at all pH levels were similar for all cultures, indicating that only the lowest pH used was detrimental to acid production for the majority of cultures studied. The data (Table 5) further emphasize the fact that there is no relationship between bacterial numbers and

acid production, in that a culture with a low maximum number of viable organisms may have a high concentration of acids.

Evidence that the ability to multiply and the ability to produce acids is not necessarily related to pH of the medium is in agreement with Mitchell's hypothesis *(16)*, that the effect of pH on growth of bacteria must presumably be a combination of the effect upon viability and upon the production and activity of the enzyme systems responsible for the growth and division of viable cells. Similarly, the ability to produce acid may be independent of the enzyme systems responsible for the growth of viable cells.

These results differ from those of other workers *(5, 11)* who, using resting cells, reported the pH range for propionic acid production to be 4.5 to 5.5, with the optimum at pH 5.2.

SUMMARY

A study was made of the effect of strain, pH, carbon source, and intermediate fermentation products on the propionic and acetic acid production of ten strains of *P. shermanii*. The organisms were grown in broth media at 30° C. for varying periods of time.

All strains of *P. shermanii* studied displayed high activity, with the exception of one, which was definitely atypical and was not considered in the results. Results revealed wide differences in the relationship of the age of culture to maximum number of organisms. There was no direct relationship between number of organisms and amount of acid produced.

The effect of initial pH at 6.6, 5.8, 5.4, and 5.2 varied somewhat with the strain used. Four of the five strains grew better at the higher pH levels. In general, a similar amount of acid was produced at each pH level but at a slower rate at lower levels.

Increasing the amount of lactose from 0.5% to one per cent resulted in an increase (60 to 111%) of total acid production of two strains. The amount of acid produced from media containing either 1.0% lactose or one per cent glucose was almost equal after six days.

Media containing lactate greatly stimulated acid production of all strains of *P. shermanii*, with maximum acid production being at two days, in contrast to eight days for the lactose media. In general, the total acid production at two days in the lactate media approximated that produced at eight days in lactose media.

Lactate media yielded five to six times more total acids than media containing the succinate and twice as much as those containing the pyruvate. Three to four times more total acid was produced in the pyruvate, than in the succinate, medium. It was suggested that lactate may be a direct precursor of the propionate.

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TECHNICAL NOTES

A COMPARISON OF FERTILITY OF BOVINE SEMEN SHIPPED IN PLASTIC AND IN GLASS CONTAINERS

Disposable plastic vials have advantages over glass tubes as containers for mailing and transporting extended semen. For example, plastic vials can be machine-labeled with permanent ink, seldom if ever break during shipment, and do not require the time and labor of washing and sterilization for re-use. Many organizations routinely ship extended semen in plastic vials, although there are no reported experimental data to indicate whether or not plastic and glass containers have the same effect on fertility. The purpose of this paper is to report the fertility results of a large number of inseminations made with extended semen shipped in glass tubes and in plastic vials.[1]

A field experiment was conducted at this station during May and June, 1956. Semen from 17 Holstein bulls in the routine shipping schedule was extended in 50% yolk-citrate containing 0.3% sulfanilamide and 500 units each of streptomycin and penicillin per milliliter. The 17 bulls, collected at weekly intervals, served as whole plots in a split-plot design. Each whole plot was divided into two subplots, for random assignment of the two shipping containers. There were two replicates of each whole plot. For example, semen from Bull 4 was shipped in plastic the first week (Subplot 1) and in glass the second week (Subplot 2) for the first replicate of Whole Plot No. 4. In the second replicate, semen was shipped in glass the third week (Subplot 1) and in plastic the fourth week (Subplot 2.) Similarly, the shipping containers were randomly assigned to each set of whole plots. The split-ejaculate technique was not used, because of the small estimated variance component for ejaculates (*1*).

Before the experiment was started, the estimate of the number of services required to detect a treatment difference of two percentage units at the 80% confidence level (probability of a Type II error=0.20) was 8,000 per treatment (*1*). The expected number of first services for each shipping method, based on the season of year and the number of bulls in the experiment, was 10,000. Therefore, two replicates were considered sufficient. The results of the experiment are shown (Table 1).

TABLE 1

Fertility of bovine semen shipped in plastic and in glass containers

Shipping container	First services	60- to 90-day N. R.
	(No.)	*(%)*
Plastic	11,289	72.5
Glass	11,762	71.7

The difference of 0.8 percentage unit in favor of plastic vials is not statistically significant ($P<0.05$). These results suggest that a conversion from standard glass shipping tubes to plastic vials, for routine shipment of extended semen, would have no adverse effect on fertility.

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H. O. DUNN

R. M. WELKER

New York Artificial Breeders' Co-op., Inc. Ithaca, N. Y.

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[1] The plastic vials were manufactured and supplied by Insemikit Co., Baraboo, Wisconsin.

THE MILLIPORE FILTER FOR ENUMERATING COLIFORM ORGANISMS IN MILK[1]

The presence of viable coliform organisms may be used in the dairy industry as an index of improper processing, inadequately cleaned or sterilized equipment, or careless handling of pasteurized milk. Although the presence of these organisms does not necessarily indicate a health hazard, it should be regarded as an indication of potential danger.

In all procedures suggested in the tenth edition of the *Standard Methods for the Analysis of Dairy Products*, by which coliform or-

[1] Contribution No. 313 from the Department of Food Technology. Based upon a paper presented at the 35th Annual Meeting of the Vermont Dairy Plant Operators' and Managers' Association, October, 1956.

ganisms in milk may be enumerated, it is difficult, if not impossible, to analyze a ten-ml. sample of milk at one time. If ten ml. of milk is to be tested, one ml. is plated in each of ten plates, or 3.3 ml. in each of 3 plates, the coliform count per ten ml. being the total number of coliform colonies on all plates.

The Millipore Filter (MF) technique has become well known in the field of bacteriology, particularly in water bacteriology. All the coliform organisms from a large volume of water sample may be concentrated and grown on one filter surface. Thomas and Woodward (*3, 4*), Eliassen (*1*), and Rawn and Bowerman (*2*) have concluded that the MF technique is as accurate as, and perhaps more precise than, the most-probable-number procedure used today in water bacteriology. Certainly, its labor- and time-saving advantages are unchallenged.

To date, the application of the MF technique to the dairy industry has been limited, owing to the fact that there has been no method for passing whole milk through the membrane. The present note describes a method which overcomes this difficulty, and makes it possible for ten ml. of milk to be analyzed at one time.

Experimental Methods

Preparation and sterilization of equipment. Fifty-ml. portions of 0.1% Triton X-100 were placed in large test tubes or small flasks and autoclaved at 121° C. for 15 minutes. The solution was found to be cloudy immediately after autoclaving, but became clear again upon cooling.

Filtering procedure. Fifty ml. of the 0.1% Triton X-100 solution at 45° C. was then poured into the funnel of the assembled apparatus and the milk (ten ml.) or cream (one ml.) was pipetted into the funnel concurrently with, or directly after, the Triton X-100 solution. A strong vacuum was immediately applied. After the liquid had passed through the filter, the funnel was rinsed thoroughly with 30 to 40 ml. of sterile rinse water. The rinse water was not used until all the sample had been filtered; otherwise, the filter became clogged.

The filter was placed on a nutrient pad in an MF disposable plastic Petri dish that contained 1.8 ml. of MF-Endo medium.[2] The Petri dish and its contents were then incubated upside down for 15 to 18 hours. After incubation, the membrane was removed from the nutrient pad and placed on a clean, dry, absorbent pad, where it was allowed to dry for five or ten minutes. The coliform colonies exhibited a golden-metallic sheen under direct light. Most colonies exhibited the sheen before the filter was dried, but the drying process occasionally revealed a few additional sheen colonies.

The experiments were at first confined to the Millipore (Hydrosol Assay) Filter. This is the type most commonly used for the detection of bacteria in relatively clear liquids. However, at most only two to three ml. of milk, in combination with the wetting agent, could be filtered. A new filter designated DA (Dairy Assay) was made available by the Millipore Filter Corporation. The DA filter has surface characteristics that will allow ten ml. of milk or one ml. of heavy cream to be filtered, if accompanied by the heated Triton X-100 solution.

In a preliminary study with *Escherichia coli*-inoculated milk it was found that the described method, besides being much more rapid, gave results which compared favorably with those obtained with the desoxycholate agar method.

C. W. Fifield
J. E. Hoff
B. E. Proctor
Department of Food Technology
Massachusetts Institute of Technology, Cambridge

[2] Formulated by the Millipore Filter Corporation.

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HYDROGENATION VERSUS CHROMATOGRAPHY IN THE ESTIMATION OF TOCOPHEROL IN CALF PLASMA[1]

In the Emmerie-Engel reaction for the estimation of tocopherol in blood plasma, interference is due principally to carotenoids (*1*), and these apparently can be eliminated by either selective hydrogenation (*1, 6-9*) or chromatography (*2, 9*).

Data, with which to base a choice between these two procedures in estimating tocopherol concentration in calf plasma, were not found in the literature. In the course of experiments conducted during 1955-1956, plasma samples from 19 calves fed varying amounts of carotene and/or vitamin A, as well as D-α-tocopherylacetate, were analyzed by two procedures, (*I*) hydrogenation (*7, 8*), or (*II*) chromatography (*2*), to determine whether differences existed between the two methods.

Although saponification is not included in the method of Quaife and Harris (*8*), it was found necessary for calf plasma, in order to eliminate turbidity in the final ethanol solutions, as was the case whether (*I*) or (*II*) was employed.

In both (*I*) and (*II*) two five-ml. samples of calf plasma in 70-ml. glass stoppered centrifuge tubes were saponified (*9*), with ten ml. of two per cent ethanolic (aldehyde-free) potassium hydroxide containing 0.5% *p*-hydroxyacetanilide, by boiling gently for 20 minutes on a steam bath. After the addition of ten ml. of water, the solutions were cooled in an ice bath, then extracted with 15 ml. of purified Skellysolve B by shaking mechanically for 20 minutes. The resulting mixtures were centrifuged for five minutes at 2,000 r.p.m. and ten-ml. aliquots of the supernatant were in (*I*) transferred to 40-ml. conical centrifuge tubes and in (*II*) to 50-ml. Erlenmeyer flasks. The extracts were evaporated under a stream of nitrogen on a steam bath.

In (*I*) the residue was taken up in ten ml. of absolute ethanol (aldehyde-free) and the resulting solution hydrogenated according to the method of Quaife and Biehler (*7*). After centrifugation, eight-ml. aliquots were transferred to Evelyn colorimeter tubes and the Emmerie-Engel reaction was read with a 520-mμ filter at the end of a 15-second interval (*8*). In (*II*), the residue was taken up in five ml. of benzene (Baker's Analyzed), transferred to a Floridin (Florex XXS, 60-90 mesh) column, and eluted with 4×5 ml. of benzene (*2*). The total eluate was evaporated to dryness under nitrogen on a steam bath, the residue taken up in ten ml. absolute ethanol (aldehyde-free), and eight-ml. aliquots read in the same manner as (*I*). Duplicate determinations were obtained by repeating the above procedures.

TABLE 1

Relationship of absorbancies due to tocopherol concentration in calf plasma, as determined by hydrogenation and chromatography

	Hydrogenation	Chromatography
Range of plasma carotenoid concentrations (γ/100 ml.)[a]	0 to 190	
Range of plasma vitamin A concentrations (γ/100 ml.)[a]	13.2 to 51.6	
Range of tocoperhol concentrations (mg/100 ml.)	0.030 to 0.676 (absorbancies)	
Range of values	0.0069 to 0.1245	0.0055 to 0.1213
Means	0.0501	0.0496
Standard error of the difference between means	0.0005	
Linear regression of chromatography on hydrogenation		
Intercept	− 0.0004 ± 0.0006	
Slope	+ 0.9976 ± 0.0144	
Linear regression of hydrogenation minus chromatography on hydrogenation		
Intercept	+ 0.0003 ± 0.0006	
slope	− 0.0023 ± 0.0144	
Duplicate variance	0.00000545	0.00000657

[a] Determined by Kimble procedure (*4*).

As shown (Table 1), the mean absorbancy values for both methods were essentially the same, with those obtained by the chromatographic procedure being only slightly less than those determined by the hydrogenation procedure. Regressions of chromatography absorbancies on hydrogenation absorbancies, as well as of hydrogenation absorbancies minus chromatography absorbancies on hydrogenation absorbancies, also indicated that the results obtained from the two methods were essentially the same, as evidenced by the intercept not being significantly different from zero, and the slope not being significantly different from one, in the first regression (*10*), and both the intercept and slope not being significantly different from zero in the second regression (*5*). Precision, as estimated by the variance of absorbancies associated with duplicate samples from the same calf,

[1] This study was supported in part by funds from the Chas. H. Hood Dairy Foundation, the Chas. M. Cox Co., Boston, Mass., and the American Cyanamid Co., Pearl River, N.Y.

was slightly better for the hydrogenation procedure (the coefficient of variation was 4.6% for the hydrogenation procedure and 5.2% for the chromatographic procedure); however, the "t" value, 0.521, for correlated mean squares (3), was not statistically signficant.

Essentially, the same results were obtained with various combinations of β-carotene, vitamin A, and D-α-tocopherol in pure solutions without saponification. Therefore, based on this and the above statistics with calf plasma, no firm choice between either hydrogenation or chromatography is indicated. However, hydrogenation has the advantage of simplicity and of requiring approximately one-half the time employed by chromatography.

MARTHA W. DICKS
J. E. ROUSSEAU, JR.
H. D. EATON
Storrs (Conn.) Agricultural Experiment Station

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OUR INDUSTRY TODAY

Brief Reviews of Current Topics

Radiation from Strontium-90 Not Found in Milk in Harmful Amounts

GAYLORD P. WHITLOCK, Ph.D.

Director of Health Education, National Dairy Council, Chicago, Illinois

The subject of strontium-90 in our food supply, and particularly in milk, is of considerable interest to the dairy industry and to others concerned with public health.

Our primary concern is to know the degree to which milk may be contaminated with radioactivity. But there is another interest of equal importance—that of being informed—so that we may stabilize public thinking with accurate information, to refute irresponsible "scare" stories which could affect milk consumption and national health.

With this is mind, an effort has been made to ascertain, reliably, the facts as they are presently known concerning strontium-90 in milk.

It is accepted that high doses of radiation from strontium-90 can produce harmful effects in human beings and that increased amounts of strontium-90 have been detected in our milk supply. The latter has probably occurred as a result of nuclear fall-out which reaches our soil and enters plant life, which is later consumed by animals and turns up in their milk.

But according to high authority,[1] radiation has *not* been detected in milk in harmful amounts in the United States. In fact, the radiation level from strontium-90 now reaching human beings in the United States from *all* sources has been measured, and is known to be less than one per cent of the amount of radiation which would be harmful.

The references to "all sources" of radiation means that radiation can reach, and is reaching, humans not only through milk but through other foods and nonfood sources, as well.

All foods which contain calcium may carry amounts of strontium-90 to the consumer which will subsequently reach the bone structure of the human body.

It is true that less strontium-90 is being deposited in adults, whose bones have already been formed, than in children. But the amount of strontium-90 being so transferred is negligible, in terms of presently accepted standards for measure of damage.

How can we be sure that accurate measures of the presence of radioactivity in the United States are available? Again, according to authoritative sources, it is possible to detect and measure with accuracy the presence of less than one ten-thousandth of the amount of radioactive strontium which scientists have declared safe for industrial practice.[1]

This ability for sensitive measure far exceeds our ability to detect, for example, the presence of harmful chemical agents in the air about us.

We have heard about air pollution in our cities as a possible source of lung cancer. A great deal of the significant and honest disagreement on the effects of air pollution is probably due to the absence of more precise equipment to measure the presence of harmful chemicals in the air. With regard to radioactive fall-out, we are fortunate, at least in the respect that we *do* have very precise techniques for being aware of its presence and for ascertaining to what degree it may be present.

Are there other than "man-made" sources of radiation which contribute to the measure of radioactive substances in our bodies? Yes.

And at this point we arrive at the realization that *some* radiation is essential to life. Natural external radiation from cosmic rays and other sources (including the national pastime of sunbathing) probably averages between seven and 15 "rads" in a lifetime. A "rad" is a unit of measure referring to the radiation which specifically reaches the human skeleton from any and all sources.

Reducing this, specifically, to the presence of radiation in the bones of children, we have the following authoritative conclusion: The present maximum amount of radiation detected in any child's bones would, if continually present over a 70-year lifetime, yield only two-tenths of a rad unit. In contrast to this, our

[1] Communication to National Dairy Council from Austin M. Brues, M. D., Director, Division of Biological and Medical Research, Argonne National Laboratory, Lemont, Illinois, cleared for publication by the AEC.

skeletons have *always* contained an amount of of the weakly radioactive forms of natural potassium and carbon, yielding about five times this amount in a lifetime.[1]

In addition, there are areas on earth where natural deposits of radium yield up to five rads in a lifetime for large populations in those areas—and these groups have not shown evidence of harmful effects. Add all this to the seven to 15 rads in a lifetime from cosmic rays and other sources and you find that we humans are exposed to quite a bit more radiation from other sources than we are from bomb fall-out. And the talk about strontium-90 being found in our milk supply in increasing amounts has been singularly related to radioactive fall-out from bomb tests.

From the foregoing information, it would seem we have a long way to go before the presence of strontium-90 in milk and other foods can catch up with the amounts of radioactivity to which we have long been exposed through natural sources.

This does not mean that the finest scientific talent in America will not continue to be very alert to all possible human danger from radioactivity. What it *does* mean, however, is that any uninformed hue and cry about strontium-90 in our food supply should be answered with the facts as we know them.

Inherited Defects in Cattle[1]

L. O. Gilmore

Department of Dairy Science and Institute of Genetics, Ohio Agricultural Experiment Station, Wooster

Defective calves within our total herd of breeding cattle become important under certain conditions, including heritability of the defect, freqency of occurrence, severity, possibility of cure by nutritional thereapy or surgery, and association with other traits, either deleterious or of economic importance.

With the current viewpoint that all traits are affected by both inheritance and environment (feed, housing, temperature, age, and management), it nevertheless becomes helpful to speak of inherited traits as those predominantly effected by gene action and interaction. Hair and body colors are good illustrations of such traits predominantly conditioned by gene action. Those traits affected to a large extent by any environmental stimulus would be called environmental traits. Highly infectious diseases illustrate this group.

Those traits in each group affected somewhat equally, or significantly, by both inheritance and environment might also be put into an intermediate classification, to emphasize the interaction of both sets of forces. Traits requiring high energy transformation from feed to food, such as milk production, stand as a good example of the importance of this interaction. From the standpoint of basic herd management involving both investment and operating factors, the investment is represented by the genes and gene combinations (genotypes) assembled in the herd; whereas, the proper combination of environment factors becomes the operating forces. The two are combined, each with respect to the other, for management to be successful.

[1] Published with the approval of the Associate Director, as Journal article 1-57, The Ohio Agricultural Experiment Station.

Inherited defects may be defined as those primarily caused by gene action, which impair the usefulness of the animal under normal or usual conditions of husbandry. If a trait is responsible for the failure to survive under such conditions, it falls into the classification of a lethal; regardless of the time after conception that the animal dies, or the unusual care required for survival. About 30 different genes, probably acting in pairs without special consideration of the effect of other genes, cause death either of the embryo, or of the calf shortly after birth. Another 15 genes have been implicated in scientific reports to affect specifically the fertility of either the cow or bull.

An equal number of physical weaknesses and traits discriminated against in the show ring, and type classification programs of the various breed-registry organizations, in the main are presumed to have an inherited basis and, therefore, to be caused or accentuated by gene action. The list thus accounts for approximately 60 defects affected by inheritance. The prevalence of defects is not well known. However, a survey conducted in cooperation with the Central Ohio Breeding Association gives some pertinent information. Abnormalities falling rather distinctly into 19 different categories were found to include taillessness, wry tail, and other tail abnormalities; blindness and small-eyedness; bull dog, parrot jaws, cleft palate; frozen joints, flexed pasterns, and extra limbs; hairlessness; congenital dropsy; no anus, and those born inside-out. Red in black breeds, which is considered abnormal by some breeders, was also found. Two or more of the above abnormalities have been found to occur in the same animal. Thus, some calves

are born without a tail, or anus, or eyeballs. One problem is to establish the bias in this estimate, and to determine the precise role of inheritance in causing the occurrence of defects.

The report that more abnormalities are found in dairy calves than in the young of beef cattle, swine, sheep, or other species is of considerable interest. This conclusion may be realistic, because dairy cattle are subject to closer observation and more adequate records than other domestic animals. The fact that only one is usually born at a time may cause a defective offspring to have more apparent meaning than in cases where only one in a whole litter is defective.

A propensity for high production might make the birth of a dead or deformed calf seem inconsequential to the main economic reason for keeping the cow. Regardless of this difference between classes of livestock, breeders are concerned, because any inherited trait caused by recessive genes can become disseminated widely through the use of popular bulls that carry the responsible genes. In fact, examples of this association exist in this country, to account for the present frequency of red in black breeds, and in Europe to account for certain lethals being widely disseminated. When daughters of such bulls are bred to others carrying the same gene, one-eighth (12½%) of the offspring will be afflicted (will be homozygous for the pair of recessive genes responsible). The elimination from the breeding herds of only cattle showing traits caused by recessive genes actually does but little to control the frequency with which the trait appears. The more rigid selection, that of culling the parents—at least the sire—of afflicted calves would be effective, but appears to be too drastic to be popular with cattle breeders. By and large, some method of detecting the carrier needs to be found, before much hope can be offered to the breeder, breeding organization, or breed-registry association which seeks to control the frequency of a particular gene and thus to govern to some extent the gene-pool investment.

If genes do not get eliminated with affected cattle, they may remain undetected for many generations. An inherited trait may take on considerable economic importance because the responsible genes can be, and in some cases have been, concentrated in a herd or breed through the selection process. The time when inherited defects show up varies widely. Some perhaps kill the fetus shortly after conception and consequently either go unnoticed or become manifested only as an irregular estrous cycle in the dam. Others show up later when the arrested growth or maldevelopment of certain organs or parts causes death of the fetus and subsequent expulsion. However, more defects show up at birth, or shortly after, than at any other short period of time.

The frequency of a defective trait in a particular herd has to exceed a threshold value before it becomes important in the management of the herd operation. From the standpoint of the investment, however, the occurrence of an inherited defect signals its presence in the gene-pool. If an inherited defect is possessed by a bull whose frozen semen is widely used, an increase in the frequency obviously will follow.

The severity of defects, either anatomical, physiological, or both, varies from slight to extreme. On the extreme side are those causing death in any circumstance of environment, such as in bulldog calves. Slightly less extreme are those permitting survival providing special environment is furnished. Cattle with severe shortening of the lower jaw (parrot jaw) can survive if provided harvested forage or an abundance of long grass to graze. Severely defective traits handicap the dairyman whose cattle possess them, whether he is a breeder or a commercial milk producer who raises his own replacements. Less severe traits of type are discriminated against in the show ring and are selected against by breeders, because of the higher breeding value of cattle meeting the type standards.

After inherited causes are established for defects, it may be possible to ameliorate the expression, or even the occurrence, by some treatment such as special diet or surgery. For instance, an operation might permit a calf with imperforate anus to survive. In Holland, surgical treatment of the retractor penis muscles effected a cure, operationally speaking, of bulls involved with impotenia coeundi (inability to copulate). Gilman recently suggested the possibility of a vitamin A imbalance effecting the penetrance of genes causing hydrocephalus. While studies of this sort are, to date, few in number, further investigations may extend the possibility of obtaining maximum economic advantage of cattle possessing genes for certain defects, until the breeder can eliminate the genes from the breeding herd through a long-time, planned culling program.

When one trait is genetically associated with a second trait—either because causative genes are closely linked, i.e., close together on the same chromosome or because of the pleiotropic effect of the same gene(s)—selection for one of the traits, no matter how desirable, would result in increasing the frequency with which the other trait appeared. In cases where the heterozygote for a pair of genes, one of which causes defective offspring in the homozygote, is favored in selection, there likewise would be built up an increased frequency of the gene. If the heterozygous calf carrying the gene for dwarfism can be visually detected, and is selected for because of greater compactness of beef form, the gene for dwarfism thus would be favored in selection. Such selective ad-

vantage could account for the greater increase with which dwarf calves now appear in some beef breeds, as compared to dairy breeds, because it is estimated that three cattle generations ago (15 years) the incidence of dwarfism was no greater in beef than in dairy breeds.

What of the Future?

By way of prognosis, it can be predicted that the problem of abnormalities will become greater with the application of new techniques that will come with the passing of time. The first reason is that ionizing radiations is one of the factors increasing the rate at which genes mutate. The estimated number of genes per cow is great enough to suggest that each head of cattle could possess a gene that is different (and probably undesirable) from those of the parents. These mutant genes will be able to pass on to offspring even though they did not come from the parents. An increase in mutant genes can be expected with the increased use of fissionable material in war and in peace. The second reason is the increased skill in surgery and internal medicine now available to our practicing veterinarians. Their use, along with improved husbandry, results in the saving for parenthood animals that in nature would not survive, or would be placed at a disadvantage in following through with the instinct of mating. Thirdly, breeders sometimes prefer, and therefore select, animals that carry undesirable genes. This illustrates how the frequency of a gene can be inadvertently increased, and that purebred cattle may not always be purebreeding parents.

To what, then, do these things come? How can the problem be resolved? One method is to select sires after they have been proved to be free of genes considered undesirable. Until laboratory methods become available for detecting carriers, proof can be obtained for the absence of single recessive genes expressed in either sex by mating a bull to about 20 daughters or sisters. The accuracy of the proof, however, will depend on the number of such matings made, and to obtain the degree of reliability often sought in experimental studies ($P=.05$), 30 to 35 such matings would be needed. The added expense involved would probably be greater than the value of the added accuracy, unless a particularly troublesome defect was involved. The second method is to learn to live with such genes, but to avoid letting any one undesirable gene become too prevalent in any one herd. If one sire is known to transmit an undesirable gene to half its offspring, the producer should avoid breeding its daughters to another bull carrying the same gene.

The salient point here is that either program includes a knowledge of what genes are involved, and how they react to predispose a cow undesirably. At the present time, this area of cattle breeding is engulfed in abysmal ignorance. Therefore, before any program can be started logically, a research program must be effected to find out for which abnormalities inheritance is of practical importance, and either how the responsible genes can be eliminated, or how the occurrence of the abnormalities can be prevented otherwise. If any one, or all, of the defects presented above is inherited, it is more important to have such information now than at any time in the past.

Breeders presumably want the facts, so that they can safeguard the future of their breeding program. Those responsible for selecting bulls to be used in artificial breeding want to know what their bulls transmit, and how to explain the occurrence of defective calves on the farms of their cooperators. Those buying bulls for their own herds want to know how to breed livestock that will have greater value to themselves and to their customers. Those responsible for registering livestock to become parents of the next generation are entrusted by their members and other constituent users of their service to improve the genetic potential of the breeds. By virtue of their position of leadership, breed-registry officials, breeding organization managers, and research personnel in our experiment stations have an obligation to point the way to progress. In the active cooperation of all interested organizations and breeders would seem to lie the hope for advancement.

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ABSTRACTS OF LITERATURE

W. O. Nelson, Abstract Editor

CHEESE

298. Roquefort-type cheese and processes of preparing the same. S. G. KNIGHT (assignor to Wisconsin Alumni Research Foundation, Madison, Wis.). U. S. Patent 2,786,762. 6 claims. Mar. 26, 1957. Offic. Gaz. U. S. Pat. Office, **716**, 4: 891. 1957.

Milk to be used for the manufacture of Roquefort cheese is modified by the addition of from 0.7 to 1.5 mg. of iron for each 100 ml. of milk. R. Whitaker

299. Apparatus for the packaging of cheese and the like. F. H. STARK. U. S. Patent 2,784,542. 17 claims. Mar. 12, 1957. Offic. Gaz. U. S. Pat. Office, **716**, 2: 305. 1957.

This filler is designed for placing process cheese in boxes having a tuck-in flap closure. During filling, the flaps are held out of the way by vacuum. R. Whitaker

DAIRY BACTERIOLOGY

300. Growth-promoting substances and their recovery. P. GYORGY AND R. KUHN AND F. ZILLIKEN (assignors, by mesne assignments, to American Home Products Corp., New York, N. Y.). U. S. Patent 2,786,051. 12 claims. Mar. 19, 1957. Offic. Gaz. U. S. Pat. Office, **716**, 3: 694. 1957.

A growth-promoting factor for *L. bifidus* is prepared by absorbtion from defatted, deproteinated, demineralized human milk. The factor is recovered in a highly purified form by elution from the absorbent with acetic acid. Some information as to the nature of the substance is given. R. Whitaker

301. Virucidal activity of hypochlorites, quaternary ammonium compounds, and iodophors against bacteriophage of Streptococcus cremoris. S. H. WATKINS, H. HAYS, AND P. R. ELLIKER. Ore. Agr. Expt. Sta., Corvallis. J. Milk and Food Technol., 20, 84. 1957.

A laboratory study was made to determine the effectiveness of hypochlorite, quaternary, and iodophor germicides on the destruction of a lactic streptococcus bacteriophage. A concentration of hypochlorite as low as 12.5 p.p.m. inactivated phage of *S. cremoris* within 30 seconds. A concentration of 100 p.p.m. of quaternaries was required to attain the same results. Iodophors were not effective, even in concentrations of 200 p.p.m., during 60 seconds of exposure.

It appears that sodium hypochlorite compounds are the most effective agents in controlling bacteriophage in dairy plants.

H. H. Weiser

302. Bacteriology of milk held at farm bulk cooling-tank temperatures. E. H. MARTH AND W. C. FRAZIER, Dept. of Bacteriol., Univ. of Wis., Madison. J. Milk and Food Technol., 20, 72. 1957.

Standard thermoduric and psychrophilic plate counts were made on raw milk samples from different farms and held at 36, 38, and 45° F. with periodic heating to 45° F. or 50° F. The plate counts were made daily for four days. At 36° F. there was no appreciable increase in microbial numbers. However, there was a noticeable increase in bacterial counts within one or two days. There was very little growth at 45° F.

No correlation was found between farm source of milk and bacterial growth. Summer milk was more favorable for psychrophilic organisms than winter milk. H. H. Weiser

303. Effect of vinegar on the growth of enteric bacteria in evaporated milk mixtures. J. O. BOND. Florida State Board of Health. J. Pediat., **49**, 6: 708. 1956.

Nine per cent of the dwellings in the United States are without refrigeration. This condition prevails principally in rural areas of the South and West, and in migrant labor camps. In other countries, much of the population is without home refrigeration. There is a direct connection between this fact and the high rate of infant diarrheal diseases which result in death.

The growth of *Escherichia coli* and *Shigella flexneri* in feeding formulas containing four per cent commercial vinegar was measured. The acetic acid concentration in the formulas ranged from 0.214 to 0.224%. After in-

oculation with the test cultures, the formulas were incubated at 20 and 37° C. For comparison, identical runs were made on formulas with no added acid. Plate counts made at intervals up to 24 hr., demonstrated that the vinegar exhibited a bacteriostatic effect, and in some cases the counts were reduced to near zero. It is postulated that the growth inhibitory effect is the result of more than the influence of the H ion. The acetate radical, or possibly the undissociated acetic acid, may exert a specific inhibitory effect. For this reason, and also because of the low cost and availability of vinegar, its use is recommended over lactic acid or the mineral acids. F. E. Rice

304. A simple method for detecting inhibitory substances in milk. E. B. COLLINS, Dept. of Dairy Ind., Univ. of Calif., Davis. The Milk Prod. J., **48**, 3: 48. 1957.

The author describes the procedure for a simple test for inhibitory substances, other than bacteriophage, in milk. The test, which is particularly sensitive to penicillin, and least sensitive to streptomycin, is not a platform test that can be used as a basis for accepting or rejecting milk on delivery, but it is adaptable to routine use.

An eight-week study revealed that 15% of the producers frequently contributed milk containing inhibitors, while 74% contributed positive samples only rarely, or not at all. Producer cooperation through proper educational programs should help in minimizing this problem. J. J. Janzen

DAIRY CHEMISTRY

305. Die Messung der elektrischen Leitfähigkeit als Hilfsmittel des Chemikers bei der Milchanalyse. (Measurement ol electrical conductivity as an aid to the chemist in milk analyses.) M. E. SCHULZ. Kieler Milchwirtsch. Forschungsberichte, **8**, 6: 641. 1956.

This article is a review of the literature of the various applications made by research workers, of the electrical conductivity of milk. T. Kristoffersen

306. Onderzockingen betreffende de Sammenstelling van melk. I. Schommelingen van de gehalten der melkconstituenten. (Contribution to the Chemical Composition of Milk. I. Variations in the Percentage of the Milk Constituents.) A. DE VLEESCHAUWER, A. VAN PUYVELDE, AND H. HENDRICKX, Govt. Agr. Coll. and Expt. Sta., Ghent, Belgium. Bull., **21**, 4: 689. 1956. (English Summary.)

Milk from 42 Belgian creameries, evenly distributed throughout the ten provinces, was analyzed for major constituents. The samples for analysis were representative of the entire intake of milk at the day of sampling. Samples were taken monthly over a one-year period from March, 1955, through February, 1956.

Fat was determined by the Gerber method, protein by the micro-Kjeldahl method (factor 6.38), lactose by polarimetry, and total solids by the Mojonnier method. The results show the following average composition of Belgian milk: fat, 3.46 ± 0.253; protein, 3.11 ± 0.242; lactose, 4.75 ± 0.104; ash, 0.706 ± 0.0157, total solids, 12.11 ± 0.327. T. Kristoffersen

DAIRY ENGINEERING

307. Container with sanitary end-closure. H. M. HILL (assignor to American Can Co., New York, N. Y.). U. S. Patent 2,784,894. 4 claims. Mar. 12, 1957. Offic. Gaz. U. S. Pat. Office, **716**, 2: 398. 1957.

A sanitary-type pouring spout located in one corner of the top of a Canco-type paper milk-bottle. R. Whitaker

308. Collapsible bottle carrier. L. JONES (assignor to Strongcraft Products, Inc., Los Angeles, Calif.). U. S. Patent 2,784,870. 2 claims. Mar. 12, 1957. Offic. Gaz. U. S. Pat. Office, **716**, 2: 392. 1957.

A folding carrier for six bottles, such as quart milk-bottles.

309. Continuous-flow attachment for soft-cream freezers and method employing the same. O. M. STALKUP, U. S. Patent 2,784,565. 5 claims. Mar. 12, 1957. Offic. Gaz. U. S. Pat. Office, **716**, 2: 311. 1957.

A freezer with an auger-type dasher. A tube connecting the draw-off valve with the back of the freezer permits a portion of the frozen ice cream to return to the freezer, where it mixes with unfrozen mix and produces a smoother and more homogeneous, finished product. R. Whitaker

310. Combined cream separator and flow meter. F. P. NOHNER (assignor to Kraft Foods Co., Chicago, Ill.) U. S. Patent 2,784,589. 5 claims. Mar. 12, 1957. Offic. Gaz. U. S. Pat. Office, **716**, 2: 317. 1957.

A slit in the outlet pipe, through which the cream is discharged from a standardizing separator, permits measurement of the cream flow. R. Whitaker

311. Two-flavor filling unit for ice cream cones and the like. J. B. ORRELL. U. S. Patent 2,784,681. 7 claims. Mar. 12, 1957. Offic. Gaz. U. S. Pat. Office, **716**, 2: 341. 1957.

A filling nozzle for placing two flavors of plastic ice cream in ice cream cones, one flavor surrounding a core of the other flavor. R. Whitaker

312. **Method and apparatus for making frozen confections.** J. L. REYNOLDS. U. S. Patent 2,784,566. 8 claims. Mar. 12, 1957. Offic. Gaz. U. S. Pat. Office, **716**, 2: 311. 1957.

Portions of ice cream and similar products are deposited on a table in a refrigerated zone, and moved slowly outward toward the edge by rotating the table, until they are finally discharged, frozen hard, on a conveyor belt for packaging. R. Whitaker

313. **Rapid refrigeration of foodstuffs.** J. L. REYNOLDS. U. S. Patent 2,784, 567. 2 claims. Mar. 12, 1957. Offic. Gaz. U. S. Pat. Office, **716**, 2: 311. 1957.

A description of the method of refrigerating the portions of ice cream described in Abstract 312. (Patent 2,784,566.) R. Whitaker

DAIRY PLANT MANAGEMENT AND ECONOMICS

314. **The small producer and bulk.** W. C. WELDEN, H. P. HOOD AND SONS, Boston. Milk Plant Monthly, **46**, 3: 18. 1957.

The charges that no single factor has delayed the growth of bulk-tank cooling so much as its unfairness to the small-volume producer, and that it imposes a hardship on him, are not true. In New England, the economics of bulk tank total 20.5¢ per cwt. for the direct city-plant producer and 25.5¢ per cwt. for the country-plant producer. For a producer averaging 150 lb. per day, the added income at the rate of 25.5¢ per cwt. would amortize in five years, an added investment of only about $625. An added income of about 40¢ per cwt. is needed for the 150 lb. producer, to enable him to amortize an added investment of $1,000, plus interest, over a five-year period. The calculations use no savings in labor or electricity, no salvage value for the present equipment, and give no weight to more convenient, lighter labor. Bulk-tank cooling offers an opportunity for the small producer to, lighten his work-load, become more efficient, increase his productivity, and improve quality. For all producers with higher production volume, the financial logistics appear to be indisputable.

C. J. Babcock

315. **Costs steady as gallonage booms.** L. R. STAUFFER, Warren Sanitary Milk Co., Warren, Ohio. Milk Plant Monthly, **46**, 3: 15. 1957.

The Warren Sanitary Milk Company is concentrating on new customers, instead of competing for old business. The new customers are the students in the Warren schools. For a school milk program to be successful, cold milk must be available and accessible. Instances are given where the per capita consumption of milk increased from 0.16 to 0.77 and from 0.20 to 0.92, when vendors were installed in the schools. C. J. Babcock

316. **Sales training.** C. BROWN, Knowlton's Creamery, San Antonio, Texas. Milk Plant Monthly, **46**, 3: 28. 1957.

Every effort should be made to expedite the training of all new sales people. After at least four days of experience in their territories, they should be given individual instruction in the manufacture of dairy products, and the successful conduct of route sales, including the use of company forms. The initial training can spell the eventual success or failure of the man. The use of training aids and methods is discussed. C. J. Babcock

317. **What's ahead for consumption?** T. D. LEATHERS. Milk Plant Monthly, **46**, 3: 60. 1957.

Present trends indicate that the total demand for dairy products should increase from 1¼ to 1½% per year for the next two decades.

C. J. Babcock

FEEDS AND FEEDING

318. **Die Beeinflussimg der Milchqualität durch das Futter. (The effect of the feed on the quality of the milk.)** A. ORTH, W. KAUFMANN, AND E. WEINERT. Kieler Milchwirtsch. Forschungsberichte, **8**, 6: 569. 1956.

This article is an extensive review of the literature on the effect of various feeds and feeding practices on the composition and quality of milk. Major emphasis is placed on fat content and consistency, protein content, and vitamins A, B, and D. T. Kristoffersen

HERD MANAGEMENT

319. **Vacuumized line milk releaser and washer.** D. E. GOLAY (assignor to Farmer Feeder Company, Inc., Cambridge City, Ind.). U. S. Patent 2,786.445. 7 claims. Mar. 26, 1957. Offic. Gaz. U. S. Pat. Office, **716**, 4: 809. 1957.

A pipe line–type of vacuum-operated milker is described. A milk reservoir is provided which holds the milk under vacuum until the quantity collected is sufficient to open a gravity-operated valve, and deliver the milk to a larger, refrigerated storage tank under atmospheric pressure. R. Whitaker

320. **Bulk-milk cooler.** R. D. PUSEY (assignor to Wilson Refrigeration, Inc., Smyrna, Del.). U. S. Patent 2,785,545. 2 claims. Mar. 19, 1957. Offic. Gaz. U. S. Pat. Office, **716**, 3: 569. 1957.

Details are given for the construction of a bulk-milk cooler, suitable for farm use. A motor-driven, demountable refrigeration system cools a cooling liquid, which in turn cools the walls of the milk tank. R. Whitaker

321. **Cow feeder.** A. VANDERBERG AND B. W. VANDENBERG. U. S. Patent 2,787,404. 3 claims. Apr. 2, 1957. Offic. Gaz. U. S. Pat. Office, **717**, 1: 111. 1957.

Feed is held in a bin, from whence it flows by gravity through gates to compartments accessible to the animals. R. Whitaker

ICE CREAM

322. Ice cream scoop. G. D. Barry (assignor of one-half to Glenn S. Mitchell, Annapolis, Calif.). U. S. Patent 2,786,429. 1 claim. Mar. 26, 1957. Offic. Gaz. U. S. Pat. Office, **716**, 4: 804. 1957.

A hand-operated device for forming cubical-shaped, individual portions of ice cream from bulk containers. R. Whitaker

323. Ice cream packaging for self-service merchandising. V. S. Packard, E. L. Thomas, and W. B. Combs, Univ. of Minn., St. Paul. Ice Cream Rev., **40**, 8: 56. 1957.

Experimental data indicate that exterior aluminum laminates, aluminum foil containers, or aluminum overwraps will effectively retard deterioration in the body and texture of ice cream stored for 4 to 5 weeks in the top level of ice cream cabinets, where temperature fluctuations are the greatest. The use of aluminum foils or exterior laminates in packaging ice cream also prevented shrinkage of the ice cream. Samples of ice cream from the same batch packaged in waxed fiber, polyethylene, and styrene cartons, and stored under the same conditions, showed pronounced shrinkage and marked deterioration in body and texture. The use of a foil laminate inner liner was ineffective in retarding body and texture losses or preventing shrinkage.

The protective ability of aluminum can be attributed to its excellent heat-conductivity properties and, to a lesser degree, to its ability to reflect radiant heat. The application of colored lacquers to all aluminum foil containers did not affect their protective ability.

Aluminum foil laminates and overwraps were found effective in retarding temperature rise in ice cream exposed to room temperatures. The laminates and overwraps were comparable in insulating ability to a corrugated paper sleeve. The best protection against warm-up of ice cream exposed to room temperatures was obtained from the use of a heavily insulated Jiffy bag, or a corrugated paper sleeve with a foil overwrap. W. J. Caulfield

324. Use of plastics in the ice cream industry. H. F. Robertson, New Product Engrg. Dept., Bakelite Co., A Division of Union Carbide and Carbon Corp., New York, N. Y. Ice Cream Rev., **40**, 8: 50. 1957.

The various types of plastics, their properties, and their varied applications in the ice cream industry are discussed.

Polystyrene is most widely used for ice cream packages. Its light weight, and the fact that it can be produced in a wide variety of colored as well as transparent types, makes it especially desirable as an ice cream packaging material.

Polyethylene, when produced as a film, is used as can liners for transporting milk, cream, and ice cream mix. It may also be used as a wrapping material for brick ice cream.

The vinyl plastics are widely used in the dairy industry as flexible tubing for conveying milk and ice cream mixes.

W. J. Caulfield

325. New ideas and trends in merchandising and selling through supermarkets. R. McNamara, Sealright Co., Inc., Fulton, N. Y. Ice Cream Rev., **40**, 8: 53. 1957.

Numerous suggestions for more effective merchandising of ice cream at supermarkets are listed and discussed in this article.

W. J. Caulfield

326. Company-owned dairy stores. J. A. Pritchard, Illinois Valley Ice Cream Co., Streator, Ill. Ice Cream Rev., **40**, 8: 54. 1957.

The establishment of neighborhood dairy stores is a satisfactory means of selling ice cream and milk when it is impossible to bid on or to get chain-store business. Five stores operated by the Illinois Valley Ice Cream Co. are selling from 13,000 to 25,000 gal. of ice cream per store per year, and milk sales have averaged $25,000 per year.

Factors considered to be important to success in this type of business are proper pricing, proper merchandising, low overhead, and accessibility to the customer.

W. J. Caulfield

MILK AND CREAM

327. The eight off-flavors. M. P. Dean, Univ. of Wis., Madison. Milk Plant Monthly, **46**, 3: 33. 1957.

The dairy industry should pay more attention to flavor of milk because the competition from other food products and within the dairy industry is more evident, and consumers judge the quality of a product by its flavor. Children are the best milk consumers and they detect off-flavor more quickly than most adults. In addition, methods used in the production and processing of milk have changed, and milk now is held for longer periods before it reaches the consumer. The flavors most often found in milk include those described as feed, weed, oxidized, rancid, barny and unclean, bacterial, salty, and foreign. The cause and prevention of these flavors are discussed.

C. J. Babcock

NUTRITIVE VALUE OF DAIRY PRODUCTS

328. Radiation processing of foods. R. H. Wasserman, Oak Ridge Inst. of Nuclear Studies. J. Am. Dietet. Assoc., **33**, 1: 33. 1957.

This is a general summary of the present status of radiation sterilization of foods. It has been found that sterilizing doses of gamma radiation destroy large percentages of vitamin A, ascorbic acid, and tocopherols in milk. It is estimated that ten years of further study may be required before the radiation sterilization of dairy products can be considered commercially feasible. In dairy products, as in most foods, the destruction of micronutrients is one of the main disadvantages of this type of sterilization. Induction of radioactivity in foods does not constitute a hazard. Measurements of the treated foods, with the most sensitive detectors, revealed no induced activity.

F. E. Rice

329. Certain features of the nutritional value of milk: A tribute to Gail Borden. (Borden Award address.) A. A. WEECH, Children's Hospital, Univ. of Cincinnati. Pediatrics, **19**, 2: 330. 1957.

The invention and development of canned, sweetened condensed milk by Gail Borden prior to the Civil War, and its use at that time, can be credited with a reduction in the incidence of what was then called "war edema," "camp edema," "prison edema," etc. We now know that this condition results from inadequate dietary protein. Maintenance on such a diet is accompanied by a steady decline in serum concentration of albumin, and depleted serum albumin is a major cause of the edema. The author reviews his own experiments with dogs, in which serum protein levels could be lowered or raised by varying the protein in the diet. Lactalbumin is at the top of the list of proteins, with beef serum, in "potency value" for regenerating serum albumin in depleted animals. Bovine colostrum and egg are in the second group. The third "potency value" group includes casein, cow's milk, and beef liver.

A second factor discussed by the author as contributing to human welfare is fortification of milk with vitamin D. Today, it is difficult to find a case of rickets, and most graduating medical students have never seen one. Dr. Weech stated, "I am not alone in holding the belief that the widespread practice of fortifying milk with vitamin D has been *the* most important factor. This viewpoint has been ably supported by the Council on Foods and Nutrition of the American Medical Association. At the time of the last tabulation, in February, 1955, there were listed 340 different brands of evaporated milk in this country, all of them fortified with Vitamin D. The opinion seems justified that this almost universal fortification of evaporated milk has done more than has anything else to eradicate rickets, in the very segments of the population least liable to avail themselves of what we may call the newer knowledge of nutrition."

F. E. Rice

330. Theoretical estimates of the protein requirements of children. D. M. HEGSTED, Harvard School of Public Health. J. Am. Dietet. Assoc., **33**, 3: 225. 1957.

Estimations of the minimum protein requirements for weight gain and for maintenance are tabulated for ages ranging from 0.5 month to 17.5 years. The sum of the two—requirements for weight gain and maintenance—is given as "total requirement." These theoretical estimates are noted to be comparable to reported protein retention values that have been published. Calculations of protein intake by breast-fed infants indicate that in the early months these infants receive less than the theoretical protein needed for maximum protein storage. F. E. Rice

331. Premature infant mortality. J. H. COREY, W. W. WADDELL, AND F. N. MITCHELL, Univ. of Va. Med. School, Charlottesville. J. Pediat., **50**, 3: 340. 1957.

The survival rate of premature infants, weighing 2,500 gm. or less at birth, was greatly improved by modifications of treatment made at this hospital. Observations on 974 unselected infants are included in this report. All infants weighing less than 2,200 gm. were started on lactic acid milk, and then graduated to an evaporated milk formula, before discharge from the nursery. Above that weight, the evaporated formula was given from the beginning. The feeding of premature infants poses no very real problem, and even the very small infants can be fed successfully with the various modifications of cow's milk. Other conditions carefully regulated in handling the prematures in the nursery were rigid isolation, high moisture content of the incubator atmosphere, use of oxygen only when indicated, and the prophylactic use of penicillin and vitamin K. F. E. Rice

332. Lysine requirements and lysine supplementation. S. E. SNYDERMAN, L. E. HOLT, P. M. NORTON, AND D. I. FOWLER, New York. AMA J. Diseases Children, **92**, 5: 456. 1956.

Six infants were fed synthetic diets at various levels of pure amino acids, including unessential as well as essential amino acids. In the lysine phase of the study, the minimal quantity which permitted normal growth was 90 mg. per kg. body weight per day. The data indicate that the lysine present in milk, as ordinarily fed to infants, covers the requirements for this amino acid, with an adequate margin of safety. The authors suggest that supplementation of the cow-milk formula with lysine is certainly unnecessary, and could possibly lead to amino acid imbalance, which is not without danger.

F. E. Rice

333. Body composition at varying planes of protein and mineral intake. W. M. WALLACE AND A. TAYLOR, Cleveland. AMA J. Diseases Children, **92**, 5: 466. 1956.

There has long been a controversy as to whether the higher levels of protein and minerals in a cow's-milk formula, as compared with human milk, are disadvantageous in infant feeding, or otherwise. Several investigators have observed higher retentions of nitrogen and certain essential mineral elements from formula feeding with cow's milk. Those who are apprehensive about the effect of such high retentions by a baby sometimes speak of "supermineralization," etc. In the research reported in this paper, weanling rats were allowed to double their weights on diets of variable nitrogen and mineral content simulating the types of milk used in infant feeding. Total body analyses indicated that the lean body mass of young, growing animals consuming rations of variable protein and mineral content have essentially similar composition. (The extended discussion which followed the presentation of this paper also is reported. Some important relations to the feeding of infants were debated.) F. E. Rice

334. Supplementary food composition table. O. B. Hayes and G. Rose, Harvard School of Public Health. J. Am. Dietet. Assoc., **33**, 1: 26. 1957.

Composition of dairy foods is included in these tables, with special reference to fat, essential and nonessential fatty acids, and cholesterol; as well as carbohydrate, protein, and total calories. The data are compiled from various sources. F. E. Rice

335. Water requirement in relation to osmolar load, as it applies to infant feeding. Committee on Nutrition, American Acadamy of Pediatrics. Pediatrics, **19**, 2: 339. 1957.

This report goes directly to the question of whether a cow-milk formula, with its higher level of protein and electrolyte as compared with human milk, may be detrimental to the infant because of a possible excessive load of solutes presented to the kidneys. Calculations are made, and tabulated in terms of milliosmoles per kg. per day, which an infant would be required to excrete when fed human milk, or cow's milk, with and without added carbohydrate. The data demonstrate that the normal baby's renal capacity is not exceeded in the feeding of a cow-milk formula, particularly when that formula contains carbohydrate added to provide one-third of the calories. Under other conditions, and during periods of high environmental temperature, additional water should be given the baby to compensate for losses other than renal. It is concluded that there is no more reason to protect the infant against the increased renal load from cow-milk formula feedings, than there would be in the routine administration of antibiotics, just because the infant is exposed to harmful organisms. F. E. Rice

336. Low-sodium milk. American Medical Assoc. Council on Foods. J. Am. Med. Assoc., **163**, 9: 739. 1957.

The preparation of low-sodium milk involves passing milk through an ion exchange resin in the cold (35 to 40° F.), which results in the replacement of sodium with an equivalent amount of potassium. While flavor is affected very little, there may be 50% losses in thiamine, niacin, and vitamin B_{12}, and losses of calcium and B_6 equivalent to 75% of their values in whole milk. The potassium content is increased to almost twice that of the original milk. The use of low-sodium milk is important in a low-sodium diet, because such a diet needs especially the protein, calcium, and riboflavin which such milk carries. The Council proposes a standard for low-sodium milk, and urges producers to make periodic analyses in order to assure compliance with the standard. F. E. Rice

337. Nitrogen requirements of the growing child. S. E. Snyderman, L. E. Holt, Jr., and P. M. Norton, New York Univ. AMA J. Diseases Children, **93**, 1: 26. 1957.

Two international agencies (F. A. O. and W. H. O.) have adopted cow's-milk protein as a standard of reference in establishing protein requirements for various diets. This study was undertaken to determine the minimal requirements for this reference protein, cow's-milk protein, by small infants. Two infants were used. Weight gain continued to be satisfactory on reducing the protein intake, until it fell below six per cent of the total calories, or 1.95 gm. protein per kg. of body weight. The protein intake could be further reduced to four per cent of the calories without evidence of impaired nutrition, by supplementing the diet with the nonessential amino acid, glycine. Preliminary studies have indicated that urea also may have a protein-sparing action, though proving less effective than glycine. The find ings point to the possibility of using simple, cheap nitrogenous compounds as protein-sparers, particularly in feeding programs for the so-called "underdeveloped" countries. The results tend also to correct the older assumption that cow's-milk protein must be fed to infants at higher levels than are required when human milk is fed. F. E. Rice

338. Food lipids and blood coagulation. N. F. Maclagen and J. D. Billimoria, Westminster Med. School, London. Lancet, **271**: 235. 1956.

In vitro tests showed that milk and milk products, including butter, increased the coagulability of human plasma. Lard and margarine were less active. Other animal fats and vegetable oils similarly tested did not affect clotting time. In preliminary feeding tests with humans, the ingestion of two ounces of butter caused clotting time to be grossly altered when determined four hours after ingestion. The feeding

of margarine had no effect. Preliminary studies indicate that the active ingredient is in the group of phospholipids. The authors suggest that the findings "would support previous impressions of the influence of milk products on coronary thrombosis." F. E. Rice

339. Steatorrhea related to a factor in cow's milk. M. Davidson and R. Burnstine, Cornell. AMA J. Diseases Children, **93**, 1: 45. 1957.

A three-month-old infant exhibited diarrhea from birth when fed a cow's-milk formula (form of cow's milk not reported). The condition was corrected when a change was made to a soybean preparation. Both saturated and unsaturated fatty acids increased in the stools when cow's milk was fed, a condition not usually observed in a simple allergy to milk. Feeding trials with purified milk proteins eliminated casein as a possible causative factor. Beta-lactoglobulin when fed in amounts equivalent to 1/100th of those contained in one quart of cow's milk brought on the diarrhea, as did a whey protein mixture equal to one-third the normal intake. Crystallized alpha-lactalbumin had no effect. F. E. Rice

340. A clinical approach to infantile colic. L. Breslow, Univ. of Ill. Med. School, Chicago. J. Pediat., **50**, 2: 196. 1957.

Studies were made to discover the causes of colic in 90 cases in private practice. In a large number of cases, colic symptoms disappeared, or were eased, by the elimination of cow's milk from the formula. It was found that many of these cases responded equally well to the elimination or lowering of the level of milk fat. The author points out that favorable results from the substitution of other products for cow's milk in the infant formula should not always be interpreted as indicating an allergy to milk protein. Equally favorable results might come from temporarily lowering the level of fat by using skimmed or half-skimmed milk. Of the 90 infants in the study, ten were definitely shown to be sensitive to milk fat. Other causes of colic were carbohydrate intolerance 22%, hunger or underfeeding 11%, allergy 10%, poor feeding technique 2%, psychosomatic 22%; the remainder included combinations of the above, and unclassified. There were five completely breast-fed infants in the group; in three of these cases, colic was established to be caused by underfeeding. F. E. Rice

SANITATION AND CLEANSING

341. A report on the evaluation of ball-spray cleaning of storage tanks and pickup tankers. D. A. Seiberling and W. J. Harper, Dept. of Dairy Technol., Ohio State Univ., Columbus. Milk Prod. J., **48**, 3: 17. 1957.

This study indicated the feasibility of cleaning storage tanks and bulk pickup tankers with permanently installed spray assemblies. The spray-cleaning system was shown through bacteriological evaluation to give results which were satisfactory, and which were superior to manual-cleaning operations. Further application of the principles and procedures can be made to nearly any food product tank or vat. Such recirculation techniques not only aid in reducing cleaning costs and improving cleaning results, but are susceptible to complete automatic control. This permits as precise a control of cleaning and sanitizing operations as is presently enjoyed with modern HTST pasteurization. J. J. Janzen

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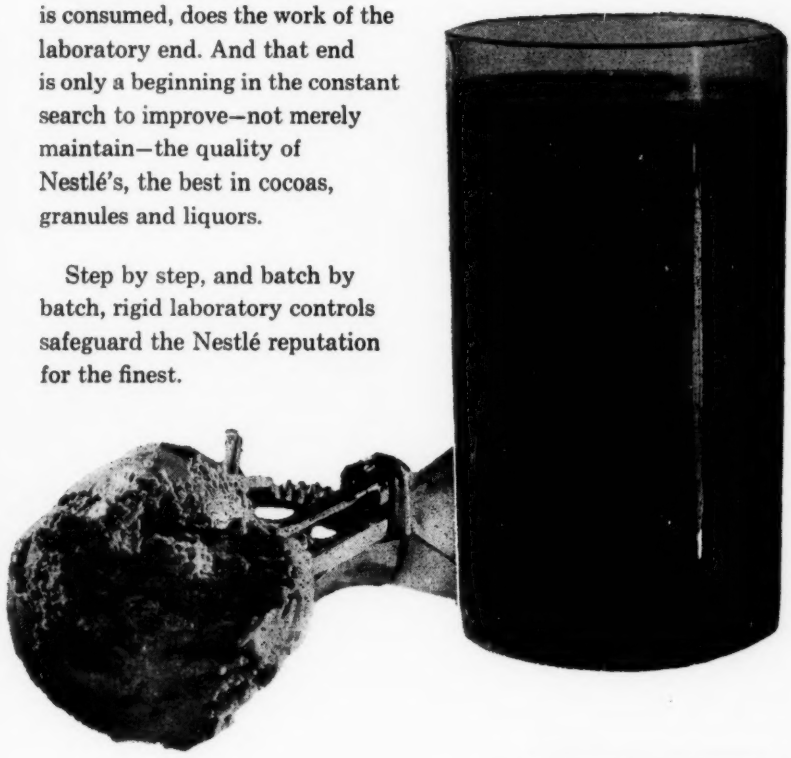

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